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DELIGNIFICATION METHODS FOR EMPTY FRUIT BUNCH CO-SUBSTRATE IN POME ANAEROBIC DIGESTION: AN EXPERIMENTAL COMPARATIVE ANALYSIS

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Abstract

Empty fruit bunch (EFB) enhances biogas production in the anaerobic digestion of palm oil mill effluent (POME) by acting as a co-substrate. Yet, lignin in EFB inhibits the performance boost. Therefore, EFB was delignified before using it as a co-substrate as much as 4%. This study compared three delignification techniques using the pairwise comparison method: Bacterial, chemical, and hydrothermal. Three parameter variations were selected for each method, namely bacterial concentration, molar concentration, and temperatures. Chemical delignification at a NaOH concentration of 2 M yielded the largest production of biogas and methane (302.0 and 153.8 mL, respectively), followed by hydrothermal at 180°C (260.0 and 83.8 mL, respectively). Although bacterial delignification required a long time (2 weeks), it was the simplest to implement and yielded the largest lignin reduction. However, the bacterial method yielded lower biogas (103-204 mL) than hydrothermal and chemical, probably because of lower hemi- and cellulose contents. Based on biogas and methane yield, production hazards, preparation time, and required infrastructure, chemical delignification was selected as the best method because of its gas production, followed closely by hydrothermal due to its efficiency and safety.

Keywords: anaerobic co-digestion, biogas, delignification, EFB, POME.

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INTRODUCTION

Indonesia and Malaysia are the world's biggest palm oil producers, with 54.0% and 32.0% of the total world's crude palm oil (CPO) production (Aziz *et al.*, 2020). In the last five years, the Indonesian Ministry for Agriculture reported an annual increase in CPO production of around 11.1% (Ditjenbun, 2021), while the Malaysian Palm Oil Board (MPOB) reported a 5.6% annual growth in the same period (MPOB, 2020).

Processing 1 t of oil palm fresh fruit bunch emits 583 kg palm oil mill effluent (POME), 210 to 230 kg empty fruit bunch (EFB), 65 kg shells, and 40 kg wet mud (Hambali and Rivai, 2017). POME is an environmental pollutant because of the high value of chemical oxygen demand (COD) between 15 000 mg O_2/L and 100 000 mg O_2/L, acidity (pH 3.4-5.2), and discharge temperatures between 80°C and 90°C (Chow *et al.*, 2020). Anaerobic-digesting lagoons are widely applied in palm oil mills before POME is

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discharged into the environment to lower their COD (Chow *et al.*, 2020).

Anaerobic digestion is an anaerobic fermentation process that involves the degradation of organic materials to produce biogas and digestate through the activities of microorganisms (Nwokolo *et al.*, 2020). 1 t of POME produces approximately 28 m^3 of biogas, consisting of methane (CH_4), carbon dioxide (CO_2) and traces of hydrogen sulfide (H_2S) (Suksong *et al.*, 2020). CH_4 is a greenhouse gas that contributes 20 times more to global warming than CO_2 (Chow *et al.*, 2020). However, its energy content can substitute fossil fuels in households and various industries.

When the process involves only one substrate, the fermentation process is called mono-digestion, while if there is more than one substrate, the process is called co-digestion. While POME is highly biodegradable due to the rich cellulolytic material and oil (Dominic and Baidurah, 2022), EFB has poor biodegradability (Liew *et al.*, 2021). However, the co-digestion of POME and EFB has proven beneficial, with EFB providing extra nutrients that increase biogas production (Chow *et al.*, 2020; Octiva *et al.*, 2018).

EFB is lignocellulosic with a large cellulose content of 24% to 65%. Other components are typically hemicellulose (21% to 34%), lignin (14% to 31%) and ash (1%) (Yiin *et al.*, 2019). The main concern with EFB utilisation in anaerobic co-digestion is the proper breakdown of the lignocellulosic substrate due to its recalcitrant and complex nature. Although hemicellulose, cellulose, and lignin are sources of nutrients for anaerobic bacteria, their decomposition rates vary wildly. Lignin, which accounts for 17% to 32% w/w in oil palm lignocellulose, is the slowest to decompose, limiting the co-digestion's performance boost (Nwokolo *et al.*, 2020).

Exposed cellulose and hemicellulose in EFB are important for efficient enzyme access (Patinvoh *et al.*, 2017). Delignification, which involves removing the lignin seal, improves the accessibility of cellulose and hemicellulose. In the case of mono-digestion, delignification enhanced methane production in waste-paper anaerobic digestion by up to 141% compared to untreated situations (Yuan *et al.*, 2012). Delignification can be performed biologically, chemically or hydrothermally (Sołowski *et al.*, 2020).

Bacterial delignification is a biological process via fungi or bacteria by their ligninolytic enzymes. Research in bacterial delignification shows significant lignin degradation in lignocellulosic biomasses (Fan *et al.*, 2018). The bacteria or fungi used in delignification are usually commercial microbial inoculants containing yeasts, lactic acid bacteria, and photosynthetic bacteria, such as *Lactobacillus* sp., *Streptomyces* sp., and *Actinomycetes* sp. (Dewi *et al.*, 2018). The biological degradation of solid organic materials consumes low energy and is environmentally and budget-friendly (Dewi *et al.*, 2018; Fan *et al.*, 2018).

Chemical delignification destroys lignin using chemicals such as NaOH. NaOH effectively degrades lignin, as was observed in previous works on EFB, wheat straw, and pine wood (Javanmard *et al.*, 2024; McIntosh and Vancov, 2011; Thong *et al.*, 2012; Salehian *et al.*, 2013).

Lastly, hydrothermal pretreatment involves hot-compressed water in a sealed pressure reactor, where water is the sole solvent (Niu *et al.*, 2022). This method has the advantage of increasing enzymes' access to lignocellulosic biomass by reducing its particle size and increasing its pore volume (Abo *et al.*, 2019). Interestingly, pre-soaking the biomass in NaOH before hydrothermal treatment resulted in even higher lignin degradation (Rahmani *et al.*, 2023; Sun *et al.*, 2021; Thong *et al.*, 2012).

The novelty of this work remains that the comparison of EFB delignification by bacterial, chemical and hydrothermal methods has never been explored in the context of providing a co-substrate for anaerobic digestion of POME. Therefore, this study compares the three methods of EFB delignification on biogas production in the anaerobic digestion of POME.

MATERIALS AND METHODS

The scope of the experiment covered EFB preparation and delignification, followed by combining POME, inoculum, and delignified EFB into different digesters. The delignification of the samples was analysed using the Chesson-Datta 1981 method (Ishola *et al.*, 2014). This method quantifies the hemicellulose, cellulose, and lignin levels within a lignocellulosic specimen. The remaining components are ash and extractive compounds.

POME's chemical oxygen demand (COD) was measured before and after digestion using the spectrophotometric closed reflux method by the Indonesian National Standard (SNI) 6989.2:2009 (Baird *et al.*, 2017; Standar Nasional Indonesia, 2009).

The resulting gas samples were sent to a Shimadzu 8A gas chromatograph-thermal conductivity detector (GC-TCD) for methane content analysis. *Table 1* shows the operational conditions for the methane content analysis.

TABLE 1. OPERATIONAL CONDITIONS FOR THE METHANE CONTENT ANALYSIS

Parameter	Notes
Apparatus	Shimadzu 8A
Detector temperature	100°C
Injector temperature	100°C

TABLE 1. OPERATIONAL CONDITIONS FOR THE METHANE CONTENT ANALYSIS (continued)

Parameter	Notes
Column type	Shincarbon ST 50-80 MESH 2.0 m x 3.0 mm I.D
Column temperature	50°C hold for 7 min (rate 20°C /min) to 100°C hold for 10 min
Gas carrier	Argon
Carrier gas flow	25 mL/min

Materials

POME was collected from a palm oil mill in Serang, Indonesia, whereas EFB was obtained from Padang, Indonesia. A methane capture plant at the same mill in Padang was the source of inoculum/starter. Inoculum is a dark, POME-based liquid which readily contains methanogenic microorganisms. It is commonly used to speed up the gas-producing bacteria's incubation in other anaerobic digestion plants.

This work employed bacterial, chemical, and hydrothermal delignification methods. An agricultural-grade bacteria labeled Effective Microorganism 4 (EM4) from PT. Songgolangit Persada was used for the bacterial process, which contains 1.09 x 10^7 CFU/mL *Lactobacillus* sp. and 4.30 x 10^7 *Saccharomyces* sp. Analytical grade NaOH (99.9%) from PT Bumi Agung Kimia was used for the chemical delignification. As for the hydrothermal method, the process used by Thong *et al.*, which pre-soaked the EFB in low-concentration NaOH (Thong *et al.*, 2012), was selected. All materials except EM4 were stored in a cooler at 4°C.

EFB Preparation

Small EFB particles were prepared to provide a large contact surface for the microbes to process the lignocellulose. Therefore, the EFB was shredded to between 2 and 5 cm before oven-drying to a maximum water content of 10% (dry basis) (Dewi *et al.*, 2018).

Bacterial Delignification

Bacterial delignification was performed aerobically to supply oxygen to the bacteria and remove the resulting toxic gases. Three perforated plastic bins were prepared as containers for the process. Dried EFB samples of 300 g each were placed in perforated plastic bins measuring 17.5 cm in diameter and a depth of 10.0 cm. The plastic bins were then sealed with hollow fabric to allow breathing while retaining the heat and moisture (Dewi *et al.*, 2018).

Three EM4 solutions of 200 mL at concentrations of 1.96%, 3.85%, and 5.66% v/v were prepared by diluting EM4 with distilled water designated as B-196, B-385 and B-566. The solutions were allowed to stand for around 12 hr. Following the work of Arbaain, each solution was sprayed evenly into the specimens and then kept for 14 days (Arbaain *et al.*, 2019). The specimens were then rinsed with distilled water through a filter cloth until the rinsing water was clear (Dewi *et al.*, 2018).

Chemical Delignification

Chemical delignification was first performed by soaking 20 g of dried EFB in NaOH solutions with variable molar concentrations of 0.50, 1.25 and 2.00 M (C-050, C-125, and C-200). The specimens were then left to stand for 10 min at ambient temperature. Next, the mixture was incubated in an oven at 100°C for 60 min, with periodic shaking every 10 min. After the incubation, the specimens were centrifuged, washed with distilled water and vacuum-filtered (Tepsour *et al.*, 2019).

Hydrothermal Delignification

Hydrothermal delignification was performed by pre-soaking the shredded EFB in a low concentration of NaOH (0.1% eq. 0.0025 M) at ambient temperature for one day. The specimens were centrifuged, washed with distilled water and vacuum-filtered (Tepsour *et al.*, 2019). 30 g samples were then treated hydrothermally using a 0.5 L autoclave, stirring at 100 rpm for 30 min in steam at 150°C, 180°C and 200°C (H-150, H-180, and H-200) (Thong *et al.*, 2012).

Drying and Pulverising

All EFB samples were oven-dried again to obtain a maximum water content of 10% (dry basis) before being milled using a high-speed pulverising machine to pass a 40-mesh screen (0.420 mm). The EFB samples were stored in airtight plastic bags at 4°C before use (Tepsour *et al.*, 2019).

Anaerobic Co-digestion

After delignification, the digesters were operated simultaneously for all methods. Erlenmeyer flasks of 500 mL were used as batch mesophilic digesters at an ambient temperature of around 27°C (*Figure 1*). The working digestion volume was set at 300 mL, and no substrates were added during the experiment. Referring to the research by Octiva, the digesters contained a mixture of POME/EFB at a ratio of 35:1 volume/mass.

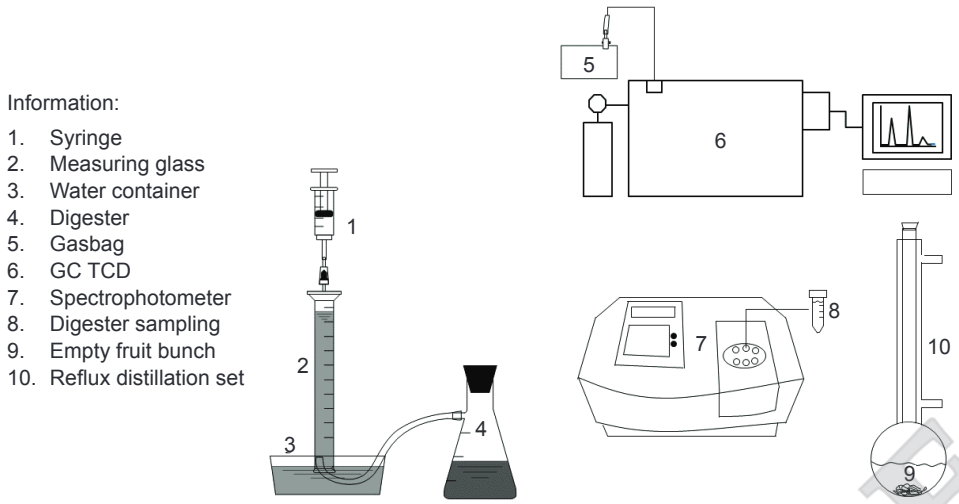

Figure 1. Experimental setup of anaerobic co-digestion.

Two control digesters were prepared: O-01 mono-digested POME and O-02 co-digested POME with untreated EFB. Nine other digesters were filled with POME and delignified EFB, designated as C-050, C-125, C-200, H-150, H-180, H-200, B-196, B-385, and B-566. Inoculum comprised 15% v/v of the working volume (Octiva *et al.*, 2018). The biogas produced in the digester would fill the top of the measuring glass and consequently push down the water level in the measuring glass. The water displacement was recorded daily as the volume of the produced biogas. Every alternating day until day 21, the accumulated gas was suctioned from the measuring glass by a syringe and transferred to a sampling bag. The gas bags were then sent for analysis after 21 days.

The methane content was calculated using the Equations (1) and (2)

$$V_{CH4,i} = \%CH_{4,i} \cdot V_{biogas,i} \quad (1)$$

$$sCH_{4,i} = \frac{V_{CH4,i}}{COD_{red,i}} \quad (2)$$

Where $V_{CH4,i}$ is the methane production of method i in mL, $\%CH_{4,i}$ the methane content of method i in %, $V_{biogas,i}$ is the biogas production of method i in mL, $sCH_{4,i}$ is the specific methane of method i in mL/g COD reduction, and finally $COD_{red,i}$ is the reduction of COD of method i in g COD reduction.

Pairwise Comparison

The delignification methods were evaluated using the pairwise comparison method (Fürnkranz and Hüllermeier, 2011). Comparison of the delignification methods was subjected to several objectives: Biogas production, methane content, preparation time, preparation requirements, and preparation hazards. Each objective was appraised and given a mark on the scale of 1 to 5. The details of the five-point scale are shown in *Table* 2.

TABLE 2. FIVE-POINT SCALE

Scale	Description
1	Very poor
2	Poor
3	Satisfactory
4	Good
5	Very good

RESULTS AND DISCUSSION

Lignocellulosic Analysis

All delignification methods showed similar visuals. EFB was initially light brown, rough, smelled of oil, and was irregularly sized. It transformed from rough to smooth after the final oven drying, resembling a sample of 90% ripeness from the study of Indriati *et al.*, 2020. The fine texture shall enable the anaerobic microbe to decompose the lignocellulose more easily (Indriati *et al.*, 2020). EFB's colour changed from light to dark brown, caused by intermediate products from lignin degradation (Poh *et al.*, 2010; Rodriguez-Yupanqui *et al.*, 2023). The processes also changed EFB's smell from oil to earthy, which might be caused by accelerated decomposition, conforming to Fan *et al.* (2018).

Similar to the study of delignification also made the EFB easier to pulverise and sieve to 40 mesh (Dewi *et al*., 2018).

Table 1 shows that delignification reduced the lignin in all samples between 5.23% (C-050) and 50.51% (B-566). Biological delignification showed noticeably higher lignin reduction than both other methods. The hemicellulose was also reduced across all samples between 5.57% (H-150) and 78.30% (C-200). Chemical delignification performance was far superior in reducing hemi than other methods, followed by biological and hydrothermal. Cellulose increase is observable, where chemical delignification is again the largest among all samples.

Bacterial Delignification (BDP)

Bacterial delignification degraded the lignin between 27.00% and 51.00%, with maximum degradation in B-566 of 50.51%. The rising degradation trend was probably caused by higher EM4 concentrations having larger populations of *Actinomycetes* sp., thus producing more peroxidase extracellular enzymes. Peroxidase enzymes accelerate the bacteria in depolymerising and solubilising the lignin content (Dewi *et al*., 2018).

The results also show that hemicellulose degraded between 11.00% and 29.00%, with B-385 as the maximum at 29.18%. Hemicellulose's amorphous and branched structure makes it susceptible to EM4 microorganisms biological hydrolysis (Dewi *et al*., 2018; Fan *et al*., 2018). Decreases in lignin break hemicellulose bonds with cellulose, yielding an increment in hemicellulose.

It should be noted that B-385, not B-566, was the highest in hemicellulose reduction and cellulose increase. This trend was probably because the biological pretreatment used microorganisms (mainly fungi) to degrade lignin and hemicellulose, but the microorganisms left the cellulose intact (Xu *et al*., 2021). The whole composition of the lignocellulose was altered, keeping the cellulose from increasing at higher bacterial concentrations.

The cellulose increased between 45.00% and 65.00%, with B-385 as the maximum at 64.68%. The increase might be caused by trapped cellulose between lignin and hemicellulose before delignification. The losses in lignin and hemicellulose released and exposed the cellulose from the lignocellulosic barriers (Silva *et al*., 2021).

Chemical Delignification (CDP).

Chemical delignification reduced lignin between 5.00% and 27.00%, with the highest reduction in C-200 at 26.45%. The delignification was caused by NaOH breaking the bonds between lignin and carbohydrates. The Na^+ ions in lignin formed water-soluble phosphate salt compounds (Fengel and Wegener, 2011). The decrease in lignin relates to the concentration of NaOH, where higher NaOH concentration resulted in higher lignin degradation, conforming to the study by Wadchasit *et al*. (2020).

Hemicellulose also decreased between 41.00% and 79.00%, with C-200 again as the maximum at 78.30%. The decrease in hemicellulose conforms with the study of Fengel and Wegener (2011), which observed that higher NaOH concentrations also resulted in a higher reduction in hemicellulose. The higher reduction might be caused by hemicellulose's susceptibility to acids and base chemicals, high temperatures and solubility (Saha *et al*., 2018). Alkali pretreatment, such as NaOH, is generally known to partially break down lignin and hemicellulose (Kulshreshtha, 2022). Some studies have shown that hemicellulose can be solubilised as NaOH concentrations increase (Modenbach and Nokes, 2014). The degradation process involves the action of hemicellulases, a group of enzymes that specifically degrade hemicellulose. These enzymes, including exoglycosidases and endo-hemicellulases, target the hemicellulose structure, releasing monomeric units and oligosaccharides (Kerr and Goring, 2011).

TABLE 3. CHANGES IN THE LIGNOCELLULOSIC CONTENT BY DELIGNIFICATION

Lignocellulosic content	Initial composition (% w/w)	Final composition (% w/w)								
	Untreated	B-196	B-385	B-566	C-050	C-125	C-200	H-150	H-180	H-200
Lignin	17.58	12.67 (-27.93)	10.78 (-38.68)	8.70 (-50.51)	16.66 (-5.23)	14.36 (-18.32)	12.93 (-26.45)	14.11 (-19.74)	11.61 (-33.96)	13.33 (-24.18)
Hemicellulose	24.06	18.29 (-23.98)	17.04 (-29.18)	21.20 (-11.89)	14.07 (-41.52)	8.55 (-64.46)	5.22 (-78.30)	22.72 (-5.57)	21.75 (-9.60)	12.59 (-47.67)
Cellulose	36.50	53.20 (+45.75)	60.11 (+64.68)	59.16 (+62.08)	62.81 (+72.08)	71.52 (+95.95)	72.94 (+99.84)	56.27 (+54.16)	59.82 (+63.89)	65.19 (+78.60)

Note: ± indicates an increase or decrease in %

Cellulose increased between 72.00% and 100.00%, with C-200 again the highest at 99.84%. The extensive increase in cellulose was caused by changes in composition after the loss of lignin and hemicellulose.

Hydrothermal Delignification (HTP)

Hydrothermal delignification combined with NaOH pre-soaking yielded moderate lignin reductions between 19.00% and 34.00%. However, it could be observed that the lignin reduction peaked at 180°C (H-180) at 33.96%. Syaftika and Matsumura›s study obtained a similar trend at temperatures of 180°C and beyond (Syaftika and Matsumura, 2018). Their study showed that at 180°C, a large part of lignin was destroyed, exposing the cellulose. Lignin remains unaltered or stable when the temperature increases to 200°C and 230°C, while cellulose degrades (Syaftika and Matsumura, 2018). Also, the stable lignin percentage at temperatures higher than 180°C is likely due to the re-polymerisation or condensation of lignin droplets, known to occur at higher temperatures (Donohoe *et al.*, 2008).

With hydrothermal processes, hemicellulose degrades more as temperatures increase, jumping to 47.67% at 200°C. The jump might be caused by hydronium ions formed in the presence of water at high temperatures, resulting in an acidic medium due to the autohydrolysis of EFB bonds. Also, acetic acid is generated from the thermal separation of acetyl groups from hemicellulose. The auto-ionisation of acetic acid due to the elevated temperature and pressure pushed these chemicals into catalysts, which encouraged further hemicellulose degradation (Syaftika and Matsumura, 2018).

The cellulose content rose steadily from 54.00% to 79.00% across the experiment temperature despite the re-polymerisation of lignin. The cellulose increase of H-200 is still high at 78.60% because the high-temperature cellulose degradation only starts at 200°C (Syaftika and Matsumura, 2018).

Cumulative Biogas Production

Figure 2 shows the cumulative biogas production, measured every other day. EFB pretreatment was proven to be effective. Delignified co-digesting reactors started producing earlier than O-02, where untreated EFB was used.

O-01 started to produce biogas very early, compared to other digesters, which shows the ease of the bacteria to mono-digest than co-digest (Wellinger *et al.*, 2013). The substrate in O-01 readily provided the specific active site for methanogenic enzymes, requiring almost no time to adapt (Sołowski *et al.*, 2020). However, O-01 production slowed down after day 7 before entering the death phase on day 14. The organic nutrients of POME were depleted, resulting in the death of the anaerobic bacteria (Prado Barragán *et al.*, 2016).

All co-digesting reactors showed that biogas production steadily grew after the bacteria acclimatised with EFB. Even after O-01 died, the co-digesting reactors showed no sign of slowing down, indicating that abundant nutrients could be converted (Chow *et al.*, 2020).

At the end of the experiment, C-200 produced the largest cumulative volume of biogas, although the initial production was slower than B-566 and O-01. C-200's initial production slope is also the highest, probably caused by the lowest lignin and highest cellulose. In the anaerobic process, cellulose and hemicellulose work together as substrates to accelerate methane production. Meanwhile, lignin is more difficult to digest and inhibits the rate of methane production.

On days 2-4 of production, B-566's production slope is the steepest, probably due to the lowest

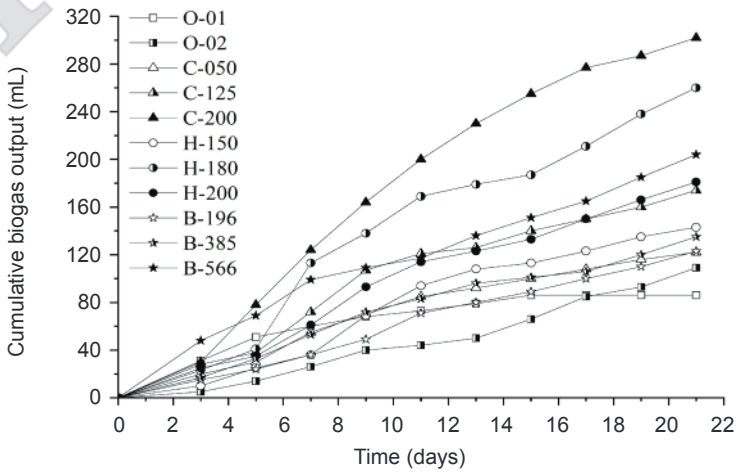

Figure 2. Cumulative biogas production (measured on alternating days).

lignin composition (Patinvoh *et al.*, 2017). The high hemicellulose content of B-566 could cause the rapid initial production of biogas. Hemicellulose decomposes faster than cellulose due to its lower degree of polymerisation molecular weight (Brodeur *et al.*, 2011; Nwokolo *et al.*, 2020). However, C-200 ultimately produced higher yield and biogas production due to the high cellulose content (72.94 w/w% of cellulose) in C-200 compared to B-566 (59.16 w/w% of cellulose).

From the production slope on day 21, it is obvious that all co-digesting reactors were still active. Adding EFB as a co-substrate causes a balance of C/N and increases the acceptable organic content to produce more biogas (Chow *et al.*, 2020).

Bacterial Delignification (BDP)

Bacterial delignification increased the cumulative biogas production by 43.00% to 137.00% compared to O-01. B-566 showed the most rapid initial output among the bacterial group reactors, most probably caused by its lowest lignin and highest hemicellulose. Hemicellulose decomposes faster than cellulose due to its low degree of polymerisation and low molecular weight (Brodeur *et al.*, 2011; Nwokolo *et al.*, 2020).

Chemical Delignification (CDP)

Chemical delignification produced 42.00% to 251.00% more biogas compared to O-01. The large spread in lignin and hemicellulose reduction probably caused the large spread of biogas production (42.00%-251.00%). C-200 had the least lignin among the chemical group digesters. Hence, it yielded the highest accumulated biogas and initial production slope.

Hydrothermal Delignification (HTP)

Hydrothermal delignification combined with NaOH pre-soaking cumulatively produced 66.00% to 202.00% more biogas than O-01. H-180 was observed to have the lowest lignin among hydrothermal group reactors. Hence, H-180 yielded the highest cumulative production and initial production slope, followed by H-200 and H-150.

Chemical Oxygen Demand (COD)

The COD value is used as a metric of the amount of oxygen needed in the chemical decomposition of organic matter. In anaerobic digestion, the COD value indicates the abundance of nutrients for the bacteria to produce biogas (Chow *et al.*, 2020). However, a high COD value is unwanted in the environment because it could kill aquatic life forms (Mohd Yusof *et al.*, 2023; Sumardiono *et al.*, 2013).

Figure 3 shows that the addition of EFB elevated the organic content, reflected in the higher initial COD values. O-01 had the lowest starting COD of 28 444 mg O_2/L, while the addition of untreated EFB in O-02 increased the initial COD to 40 927 mg O_2/L. With the addition of pretreated EFB, the chemical group averaged 41 000 mg O_2/L, the hydrothermal group averaged 38 000 mg O2/L, and the bacterial group averaged 47 000 mg O_2/L. The average initial COD of the hydrothermal group is noticeably lower than O-02. This lower value was probably caused by the lower additional organic content in hemicellulose and cellulose (*Table 3*).

Adding EFB causes a jump in COD because EFB comprises various organic compounds. Delignification further increases the COD because the lignocellulosic material is broken down into simpler sugars and organic acids (Fitrah *et al.*, 2019; Muryanto *et al.*, 2022; Octiva *et al.*, 2018)

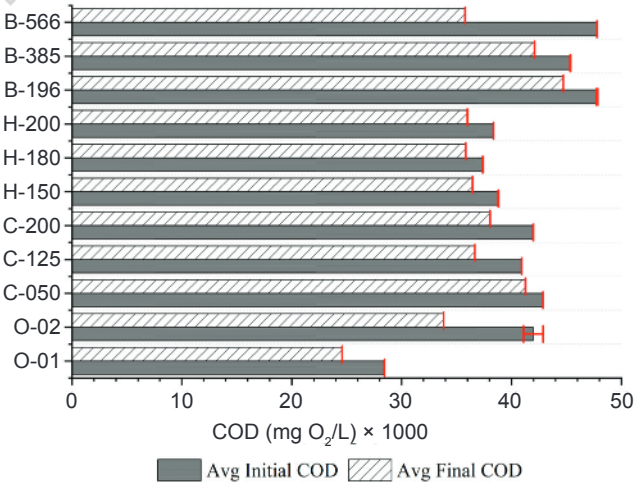

Figure 3. COD of samples before and after 21 days of anaerobic digestion.

After 21 days, the COD of O-01 was the lowest. All samples showed reductions in COD, but the co-digesters' COD was still higher than the mono-digesting O-01, indicating high contents of nutrients remained for further biogas production (Chow *et al.*, 2020). Since O-01 died on day 14, its final COD value of 24 590 mg O_2/L could measure where other digesters would stop producing biogas. O-02's COD after 21 days of 33 183 mg O_2/L is the lowest among the co-digesters.

Methane Production

Methane contents of the produced biogas are an index of the biogas quality. *Figure 4* shows the methane percentages of each reactor, where the impact of EFB addition is obvious. C-200 produced biogas with the highest quality, with 47.61% biogas with a total methane volume of 143.77 mL and COD removal of 9.37%. Interestingly, O-02 produced the second-best quality of biogas. However, its production was the slowest among the co-digesting reactors due to the time required for the bacteria to process untreated EFB.

Mono-digesting O-01 produced the lowest methane compared to the co-digesting samples. C-200 produced biogas with the highest methane after 21 days, followed by H-180 (*Figure 5*). The lignocellulosic contents of both C-200 and H-180 most likely caused the spike in methane production.

H-180 yielded the highest specific methane production (SMP), defined as the ratio of produced methane over absolute COD reduction (*Figure 6*). Thus, H-180 potentially produce the most methane when the organic contents are fully consumed. The results in the methane production of hydrothermal group reactors closely resemble the study of Thong *et al.*, where they found the optimal hydrothermal temperature of 175°C. The spike of SMP in H-180 was most probably caused by the conversion of lignin into acetic acid under alkaline conditions, which is beneficial for methanogenic microorganisms (Kaparaju and Felby, 2010; Thong *et al.*, 2012).

Production Scale-up Scenario

A typical covered lagoon for anaerobic digestion can measure 150 m by 50 m, with a depth of 7 m. If the delignified EFB ratio is kept the same as in the experiment, around 1.91 t of delignified EFB will need to be prepared. This quantity of delignified EFB would require a well-planned infrastructure.

After fresh EFB is cut into size, bacterial delignification can be performed in a covered warehouse, which would take 14 days to complete before it is washed and ground to size. On the other hand, delignification methods by chemical and hydrothermal methods can be completed within a much shorter time of 60 and 30 min, respectively.

Chemical delignification would need containers to perform the NaOH soaking and washing. A large container of around 5 m^3 can perform the soaking, and the delignification can be performed in batches until the required volume is reached. It should be noted that the spent NaOH cannot be freely disposed of after use since it is an environmental hazard; thus, it requires a recycling process.

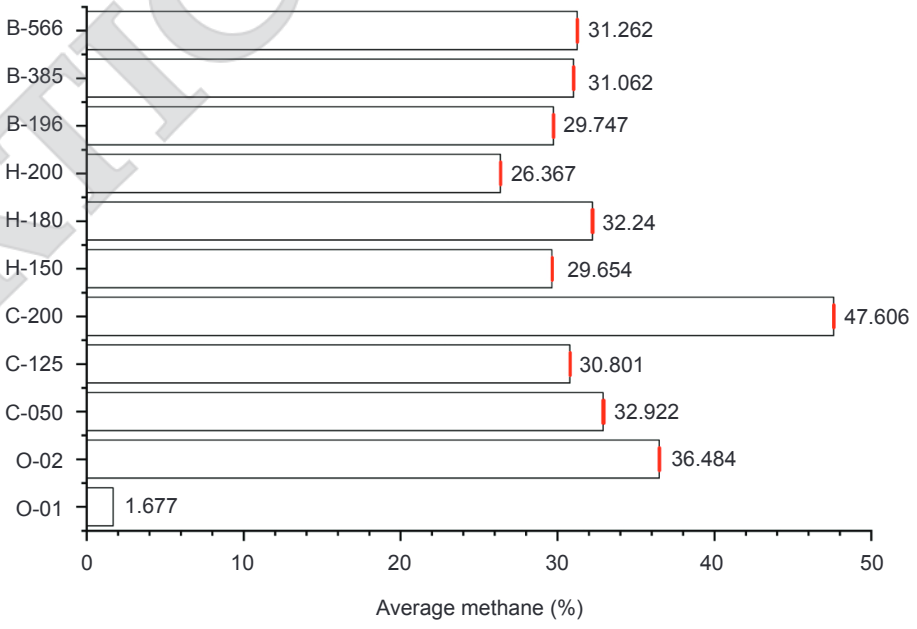

Figure 4. Methane content.

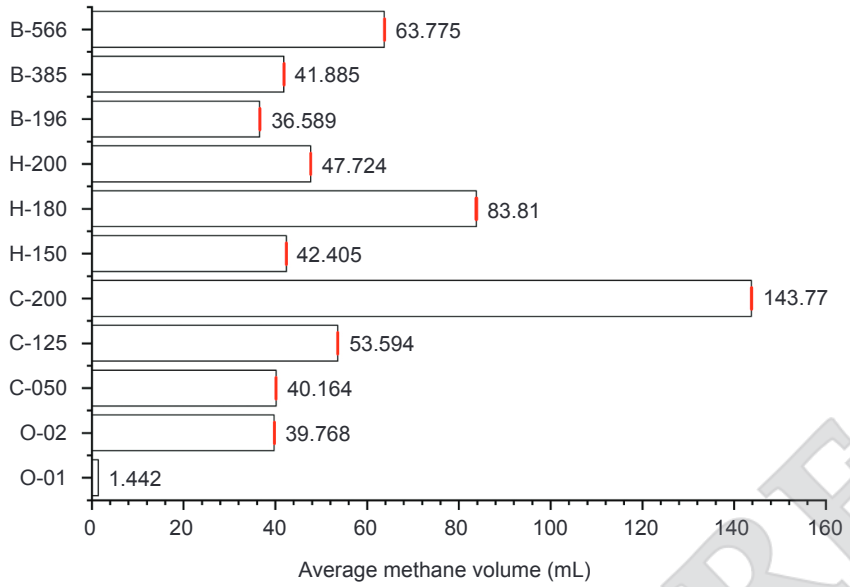

Figure 5. Methane production.

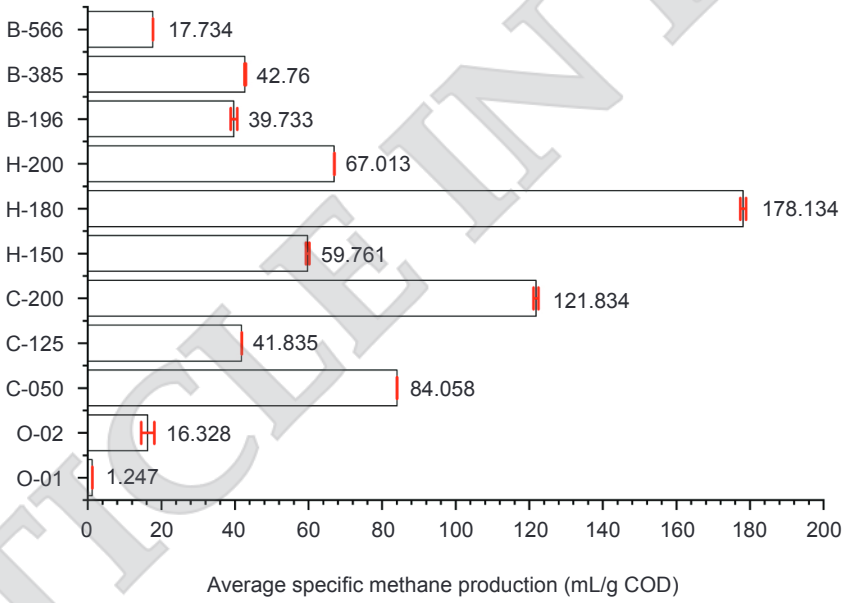

Figure 6. Specific methane production.

Hydrothermal delignification will require a small processing plant where the NaOH pre-soaking could occur and a boiler as a heat source for the hydrothermal reactor. All three methods require a grinding facility to get the delignified EFB to size.

Pairwise Comparison

A pairwise comparison arranged the level of importance of each selection objective (*Table 4*). A pairwise comparison chart was constructed by comparing each of the objectives with one another. The scoring marks for the comparison process were 0.0, 0.5, and 1.0 points, indicating the less, equal, and most favoured, respectively.

The obvious preferred objectives are biogas quality (MC) and volume (BP), with weighted scores of 0.37 and 0.32, respectively. Safety (PH) is also considered high and scored 0.21, followed by preparation time (PT) and infrastructure (PI). *Table 5* lists the objectives arranged in their respective order of importance (score) and their respective description.

TABLE 4. PAIRWISE COMPARISON CHART OF VARIOUS OBJECTIVES

Objective	BP	MC	PT	PI	PH	Score	Weight
Biogas production (BP)	-	0.50	1.00	0.50	1.00	3.00	0.32
Methane content (MC)	0.50	-	1.00	1.00	1.00	3.50	0.37
Preparation time (PT)	0.00	0.00	-	0.50	0.00	0.50	0.05
Preparation infrastructure (PI)	0.00	0.00	0.50	-	0.00	0.50	0.05
Preparation hazards (PH)	0.00	0.00	1.00	1.00	-	2.00	0.21

TABLE 5. OBJECTIVES ARRANGED BY THE ORDER OF IMPORTANCE

Rank	Objective	Description
1	MC	High methane content is desired for the quality of biogas as fuel
2	BP	High biogas production volume is much desired because it is a potential fuel source for households and small industries.
3	PH	The hazardous processes carry an inherent health and safety risk to the processing personnel and the environment.
4	PT	The preparation time of the co-substrate is important in the continuous production of biogas. However, the co-substrate can be prepared inbatch for multiple production.
4	PI	The required infrastructure to process the co-substrate is key to the efficiency of the selected delignification method.

TABLE 6. PAIRWISE SCORING OF THE DELIGNIFICATION METHOD SELECTION

Objectives	Weight	Bacterial		Chemical		Hydrothermal	
		Score	Weighted Score	Score	Weighted Score	Score	Weighted Score
MC	0.37	2.00	0.74	5.00	1.85	4.00	1.48
BP	0.32	2.00	0.64	4.00	1.28	3.00	0.96
PH	0.21	5.00	1.05	1.00	0.21	4.00	0.84
PT	0.05	1.00	0.05	3.00	0.15	5.00	0.25
PI	0.05	5.00	0.25	3.00	0.15	2.00	0.10
Score			2.73		3.64		3.63
Rank			3		1		2

The delignification methods were ranked with the weight objective method. The calculation of weighting and rating is shown in *Table 6*. Each option was appraised and marked on a scale of 1 to 5. The score was weighted through the pairwise comparison chart from *Table 4* and used to rank each method. The high methane contents of the produced biogas ranked the chemical group as the highest, with 5 points for methane content (MC). Due to the high volume of chemicals required for the chemical process, it ranked the lowest in the hazard objective (PH). On the other hand, bacterial delignification was considered the safest to operate since it is mainly a biological process. However, due to its long preparation time of 14 days (PT), biological delignification ranked the lowest, with hydrothermal delignification the highest. If the pre-soaking is performed in batches, it only takes 30 min to prepare. Due to the complex required investment and maintenance, hydrothermal delignification is ranked lowest in the infrastructure objective (PI).

The pairwise comparison shows that chemical delignification ranked the highest (3.64) due to the biogas produced and its quality. Also, it requires simple infrastructure. However, its productional hazards rendered it almost equally preferred to hydrothermal (3.63).

CONCLUSION

There is a close correlation between the EFB's lignocellulosic composition and biogas/methane production. The maximum delignification of 50.51%

was reached with bacterial B-566. However, chemical C-200 showed the highest cellulose increase of 99.84% and a hemicellulose reduction of 78.30%. Consequently, after 21 days, C-200 produced the largest volume of biogas and methane (302 mL and 153.8 mL, respectively). A pairwise comparison of the three delignification methods has concluded that chemical delignification was the most preferred method, followed very closely by hydrothermal. Chemical delignification scored the highest due to its biogas production and quality. Hydrothermal delignification was second highest because of its efficiency, safety, and modest biogas production and quality.

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