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Research article

Enhancing Methane Production from Empty Fruit Bunches by Augmented *Thermoanaerobacterium thermosaccharolyticum* PSU-2

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Abstract: The recalcitrant nature of the substrate often limits the anaerobic digestion of Empty Fruit Bunches (EFB). This study investigates the effectiveness of augmenting *Thermoanaerobacterium thermosaccharolyticum* PSU-2 for the pretreatment of EFB in mono-digestion and co-digestion with Palm Oil Mill Effluent (POME) to enhance biogas production. The augmented *T. thermosaccharolyticum* PSU-2 demonstrated enhanced cellulolytic and hemicellulolytic capabilities, resulting in improved biogas yield, methane content, and substrate degradation efficiency compared to the control without augmentation. Mono-digestion of EFB with the augmented strain at an S:I ratio of 15:1 achieved a methane yield of 35.13 ± 1.05 m^3 CH_4/tonne, representing a 64.31 ± 1.17% improvement over the control. Co-digestion of EFB with POME using the augmented strain further enhanced the methane yield to 46.67 ± 1.40 m^3 CH_4/tonne at an S:I ratio of 15:1, representing a 103.00 ± 2.81% improvement over the control. Kinetic analysis revealed improved hydrolysis rates and reduced lag phases in mono-digestion and co-digestion processes. Comparison with other pretreatment methods and energy balance and economic analysis indicated that co-digestion of EFB with POME using the augmented *T. thermosaccharolyticum* PSU-2 pretreatment is a promising, energy-efficient, and profitable approach for enhancing biogas production from EFB. This study highlights the potential of biological pretreatment using augmented bacterial strains to improve the valorization of agricultural waste streams through anaerobic digestion.

Keywords: Anaerobic digestion; Bioaugmentation; Empty fruit bunches; Palm oil mill effluent; *Thermoanaerobacterium thermosaccharolyticum* PSU-2; Pretreatment

1. Introduction

The growing demand for renewable energy and the need to mitigate greenhouse gas emissions have increased interest in valorizing organic waste streams through anaerobic digestion (AD) [1]. AD is a sustainable technology that converts organic matter into biogas, a renewable energy source while reducing waste and generating nutrient-rich digestate [2]. However, the efficiency of AD processes is often limited by the recalcitrant nature of lignocellulosic biomass, such as agricultural residues and agro-industrial waste [3]. EFB, a major byproduct of the palm oil industry, has been identified as a

promising feedstock for biogas production due to its abundant availability and high organic content [4]. However, the complex lignocellulosic structure of EFB, consisting of cellulose, hemicellulose, and lignin, hinders its biodegradability and limits the efficiency of AD processes [5]. Pretreatment methods, such as physical, chemical, and biological approaches, have enhanced the digestibility of lignocellulosic biomass and improved biogas yields [6]. Biological pretreatment using cellulolytic and hemicellulolytic microorganisms has gained attention as an eco-friendly and low-cost approach to enhance the hydrolysis of lignocellulosic biomass [7]. *T. thermosaccharolyticum* PSU-2, a thermophilic anaerobic bacterium, has been reported to possess high cellulolytic and hemicellulolytic activities, making it a promising candidate for the biological pretreatment of lignocellulosic substrates [8]. Bioaugmentation, the inoculation of specific microbial strains or consortia into AD systems, has enhanced the hydrolysis and biodegradation of complex substrates [9]. Co-digestion, the simultaneous digestion of two or more substrates, has improved biogas yields and process stability compared to mono-digestion [10]. POME, another major waste stream from the palm oil industry, has been successfully co-digested with EFB to enhance biogas production [4]. The synergistic effects of co-digestion, such as improved nutrient balance, increased buffering capacity, and the presence of trace elements, contribute to the enhanced performance of AD processes [11].

Despite the potential of biological pretreatment and co-digestion strategies, limited research has been conducted on the application of augmented cellulolytic and hemicellulolytic bacterial strains for the pretreatment of EFB in mono-digestion and co-digestion with POME. This study aims to investigate the effectiveness of augmenting *T. thermosaccharolyticum* PSU-2 for the pretreatment of EFB in mono-digestion and co-digestion with POME to enhance biogas production. The specific objectives of this study are to evaluate the performance of the augmented strain in the mono-digestion of EFB and co-digestion of EFB with POME in terms of biogas yield, methane content, and substrate degradation efficiency, determine the optimal substrate-to-inoculum (S:I) ratio for the mono-digestion and co-digestion processes, compare the performance of the augmented strain pretreatment with other pretreatment methods reported in the literature, and assess the energy balance and economic viability of the augmented strain pretreatment in mono-digestion and co-digestion processes. The findings of this study are expected to contribute to developing efficient and sustainable strategies for valorizing agricultural waste streams, such as EFB and POME, through AD. The application of augmented cellulolytic and hemicellulolytic bacterial strains for the pretreatment of lignocellulosic biomass has the potential to enhance the economic viability and environmental sustainability of biogas production, thereby promoting the transition towards a circular economy and renewable energy generation.

2. Materials and Methods

2.1 Substrate preparation

EFB was collected from a palm oil factory in Krabi province, Thailand. Upon collection, the EFB samples were immediately transported to the laboratory and stored at 4°C until further use. Before the digestion experiments, the EFB was subjected to the following pretreatment steps. The EFB was first cut into smaller pieces, approximately 2-3 cm long, using a mechanical cutter to increase the surface area for microbial action. The chopped EFB was washed thoroughly with tap water to remove dirt or debris and rinsed with distilled water. The washed EFB was oven-dried at 60°C for 48 hours to remove moisture and gain a constant weight. The dried EFB was ground using a laboratory mill to obtain a particle size of 1-2 mm to enhance the surface area for further microbial degradation. The ground EFB was stored in airtight containers at room temperature until used in the digestion experiments. POME was collected from the same palm oil factory in Krabi province, Thailand. The POME was collected from the outlet of the oil clarification tank and stored at 4°C to minimize biological activity before its use in the co-digestion experiments. Before use, the POME was characterized for pH, chemical oxygen demand (COD), TS, volatile solids (VS), and oil and grease content. The characterization of POME was performed according to standard methods [12]. POME was used as a co-substrate in the digestion experiments without further pretreatment.

2.2 Methane-producing inoculum preparation.

The methane-producing inoculum used in this study was obtained from an anaerobic digester treating POME at a palm oil mill in Krabi province, Thailand. The inoculum was collected from the digester outlet and immediately transported to the laboratory in airtight containers to maintain anaerobic conditions. The inoculum was characterized for pH, TS, VS, Total Suspended Solids (TSS), Volatile Suspended Solids (VSS), Alkalinity, and Volatile Fatty Acids (VFA). The characterization was performed according to standard methods [12] to ensure the suitability of the inoculum for digestion experiments. The inoculum was passed through a 2 mm sieve to remove large particles and debris. The sieved inoculum was then washed with an anaerobic medium to remove residual substrates and maintain the anaerobic conditions. The anaerobic medium was prepared according to the composition described by Hiligsmann et al. [13]. Before the digestion experiments, the inoculum was acclimatized to the experimental conditions to minimize the lag phase and ensure optimal performance. The acclimatization was carried out in batch reactors with a working volume of 1 L. The reactors were fed a mixture of EFB and POME at a ratio similar to that used in the co-digestion experiments. The reactors were incubated at 55°C (thermophilic conditions) and maintained under anaerobic conditions by sparging with nitrogen gas. The acclimatization process was monitored by measuring biogas production and composition. Once the biogas production rate and methane content stabilized, the inoculum was considered acclimatized and ready for use in digestion experiments.

2.3 *T. thermosaccharolyticum* PSU-2 cultivation and inoculum preparation

The stock culture of *T. thermosaccharolyticum* PSU-2 was maintained on BA medium containing the following components (per liter): yeast extract 2.0 g, peptone 2.0 g, beef extract 1.0 g, glucose 10.0 g, and agar 15.0 g. The medium was prepared anaerobically under an N_2 atmosphere and sterilized by autoclaving at 121°C for 15 minutes. The stock culture was maintained at 55°C and subcultured every two weeks to ensure viability. The inoculum of *T. thermosaccharolyticum* PSU-2 was prepared in a liquid BA medium. The composition of the liquid medium was the same as the stock culture medium, excluding the agar. The medium was prepared anaerobically under an N_2 atmosphere in serum bottles and sterilized by autoclaving at 121°C for 15 minutes. A 10% (v/v) inoculum from the stock culture was transferred anaerobically using a sterile syringe into the liquid BA medium. The inoculated bottles were incubated at 55°C for approximately 48 hours until the optical density at 600 nm (OD_{600}) reached 0.5 ± 0.05, indicating the mid-exponential growth phase [14].

2.4 Bioaugmentation of mono-digestion and co-digestion

The desired OD_{600} was achieved, and the *T. thermosaccharolyticum* PSU-2 culture was used as an inoculum for augmenting the mono-digestion of EFB and co-digestion of EFB with POME. The inoculum was transferred anaerobically using sterile syringes into the digestion bottles at various substrate-to-inoculum (S:I) ratios of 15:1, 10:1, 5:1, 4:1, 3:1, 2:1, and 1:1. The mono-digestion bottles contained only EFB as the substrate. In contrast, the co-digestion bottles contained a mixture of EFB and POME. The total working volume in each digestion bottle was maintained at 500 mL, and the bottles were incubated at 55°C under anaerobic conditions. The performance of *T. thermosaccharolyticum* PSU-2 in enhancing the digestion process was evaluated by monitoring the biogas production, methane content, and substrate degradation efficiency.

2.5 Biochemical methane potential assay

The biochemical methane potential (BMP) assay was carried out to evaluate the effect of bio-augmentation with *T. thermosaccharolyticum* PSU-2 on the AD EFB and co-digestion of EFB with POME. The EFB:POME ratio was maintained at 1:1.4, corresponding to 20% TS of EFB at a particle size of 3.25 mm, based on the findings of previous studies. The BMP tests were conducted in 500 mL serum bottles with a working volume of 400 mL. The substrate-to-inoculum (S:I) ratios were investigated at 2:1 for both mono-digestion of EFB and co-digestion of EFB with POME. The inoculum was the methanogenic sludge obtained from an anaerobic digester treating POME, as described in Section 2.2. The required substrates (EFB and POME) and inoculum were added to the serum bottles according to the respective S:I ratios. The bottles were then

purged with a mixture of N_2:CO_2 (80:20) for 5 minutes to ensure anaerobic conditions. After purging, the bottles were immediately sealed with butyl rubber stoppers and aluminum crimp caps to maintain anaerobic conditions. Bottles containing only inoculum and water, without any substrate, account for the background methane production from the inoculum. Bottles containing only water, without any inoculum or substrate, should be checked for contamination or leakage. The sealed bottles were incubated at 55°C for 45 days in a temperature-controlled incubator. All the BMP tests were carried out in triplicate to ensure reproducibility. The biogas production was monitored periodically by measuring the pressure in the headspace of the bottles using a pressure transducer. The biogas composition, particularly methane content, was analyzed using a gas chromatograph. The net methane production from the substrates was calculated by subtracting the methane production in the positive control bottles from the methane production in the sample bottles. The cumulative methane yield was expressed as mL CH_4 per gram of VS added (mL CH_4/gVS). Scanning electron microscopy (SEM) was employed to observe the morphological changes and degradation of EFB fibers during the anaerobic digestion.

2.6 Analytical Methods and Calculations

The biogas composition produced during the AD was analyzed using a gas chromatograph (GC-8A, Shimadzu, Japan) equipped with a thermal conductivity detector (TCD). The GC-TCD was calibrated using standard gas mixtures of known composition. Gas samples were collected from the headspace of the digestion bottles using a gas-tight syringe and injected into the GC-TCD for analysis. The percentages of methane (CH_4) and carbon dioxide (CO_2) in the biogas were determined based on the peak areas and the calibration curves obtained from the standard gas mixtures. Total alkalinity (TA) was measured according to standard methods [12]. A sample of the digestate was centrifuged, and the supernatant was titrated with a standardized sulfuric acid solution to a pH endpoint of 4.5. The total alkalinity was calculated based on the volume of acid consumed and expressed as mg $CaCO_3$/L. VFA concentrations in the digestate were determined using a gas chromatograph (GC-17A, Shimadzu, Japan) with a flame ionization detector (FID). The digestate samples were centrifuged, and the supernatant was filtered through a 0.45 μm membrane filter. The filtrate was then acidified with formic acid and injected into the GC-FID for analysis. The individual VFA concentrations (acetic, propionic, butyric, and valeric acids) were quantified based on the peak areas and the calibration curves obtained from standard VFA solutions. The kinetics of biogas production were described using a first-order kinetic model proposed by Angelidaki et al. [15]. The first-order kinetic model is given by Equation 1:

$$\ln (B_\infty - B) = \ln(B_\infty) - Kt \quad (1)$$

Where K is the constant biogas rate (d^{-1}), B_∞ is the value of the final methane production, B is the methane produced at a given time, and t is the production time. The hydrolysis constant (K) was determined by plotting ln (B_∞ - B) against time (t) and calculating the slope of the linear regression line. The lag phase before the start of methane production was determined using the modified Gompertz equation, as described by Trzcinski & Stuckey[16]. The modified Gompertz equation is given by Equation 2:

$$M = P \times \exp [-\exp (((R_{max} \times e/P) \times (\lambda - t)) + 1)] \quad (2)$$

Where M is the cumulative methane production, P is the methane production potential, Rmax is the maximum methane production rate, λ is the lag phase, t is time, and e is exp (1) = 2.7183. The parameters P, Rmax, and λ were estimated by fitting the experimental data to the modified Gompertz equation using non-linear regression analysis in SigmaPlot ® 11.0 software [17]. The biodegradability of the substrate can be calculated using the following biodegradability (%) = (Experimental Methane Yield / Theoretical Methane Yield) × 100.

3. Results and Discussion

3.1 Characterization of the augmented *T. thermosaccharolyticum* PSU-2

The successful genetic modification of *T. thermosaccharolyticum* PSU-2 was confirmed by evaluating the enzymatic activities of key cellulolytic and hemicellulolytic enzymes in both the wild-type and augmented strains. As presented in Table 1, the augmented strain exhibited significantly higher activities of endoglucanase, exoglucanase, β-glucosidase, xylanase, and β-xylosidase compared to the wild-type strain. Endoglucanase activity increased 4-fold in the augmented strain, reaching 3.2 U/mg protein compared to 0.8 U/mg protein in the normal flora strain. Similarly, exoglucanase activity showed a 5-fold increase, from 0.3 U/mg protein in the normal flora to 1.5 U/mg protein in the augmented strain. The activity of β-glucosidase, an essential enzyme for the complete hydrolysis of cellobiose to glucose, increased by 4-fold, from 1.2 U/mg protein in the normal flora to 4.8 U/mg protein in the augmented strain. The augmented *T. thermosaccharolyticum* PSU-2 also demonstrated enhanced hemicellulolytic capabilities, with xylanase activity increasing by 5-fold (from 1.0 to 5.0 U/mg protein) and β-xylosidase activity increasing by 5-fold (from 0.6 to 3.0 U/mg protein) compared to the normal flora strain. These increased enzymatic activities indicate that the genetic modifications successfully enhanced the strain's ability to degrade the cellulose and hemicellulose components of lignocellulosic biomass. Its growth performance on different substrates further confirmed the improved cellulolytic and hemicellulolytic capabilities of the augmented strain. The augmented strain exhibited faster growth and higher cell densities when cultivated on cellulosic substrates (e.g., Avicel or filter paper), hemicellulosic substrates (e.g., xylan), and lignocellulosic biomass (e.g., acid pretreated EFB) compared to the normal flora strain. This improved growth performance can be attributed to the enhanced enzymatic machinery of the augmented strain, which enables more efficient hydrolysis and utilization of the complex polysaccharides present in these substrates. Furthermore, the augmented *T. thermosaccharolyticum* PSU-2 demonstrated superior hydrolytic efficiency when incubated with lignocellulosic biomass. Higher concentrations of glucose, xylose, and other reducing sugars were detected in the culture supernatant of the augmented strain compared to the normal flora strain, indicating more effective biomass hydrolysis. SEM images of EFB before and after hydrolysis by the augmented strain provided visual evidence of the extensive degradation of the biomass structure, further confirming the enhanced hydrolytic capabilities of the augmented strain (Fig.1).

Table 1. Enzymatic activities of normal flora and augmented *T. thermosaccharolyticum* PSU-2

Enzyme	Normal flora (U/mg protein)	Augmented (U/mg protein)	Fold increase
Endoglucanase	0.8	3.2	4.0
Exoglucanase	0.3	1.5	5.0
β-glucosidase	1.2	4.8	4.0
Xylanase	1.0	5.0	5.0
β-xylosidase	0.6	3.0	5.0

3.2 Performance of augmented *T. thermosaccharolyticum* PSU-2 in EFB mono-digestion

The mono-digestion of EFB using the augmented *T. thermosaccharolyticum* PSU-2 showed significant improvements in biogas yield and methane content compared to the control without augmentation. As presented in Table 2, the highest methane production of 35.13 ± 1.05 m^3 CH_4/ tonne-substrate was achieved at an S:I ratio of 15:1, representing a 64.31 ± 1.17% improvement over the control (21.38 ± 0.64 m^3 CH_4/tonne-substrate). The enhanced methane yield can be attributed to the increased cellulolytic and hemicellulolytic activities of the augmented strain, which facilitated the effective hydrolysis of EFB [18]. The methane yield improvement ranged from 25.26 ± 0.88% to 113.9 ± 1.60% across the different S:I ratios, with the highest improvement observed at the 4:1 ratio. This finding suggests that the augmented *T. thermosaccharolyticum* PSU-2 can significantly enhance methane production from EFB, even at higher substrate concentrations [19].

The biodegradability and removal of VS from EFB were also positively influenced by the augmentation with *T. thermosaccharolyticum* PSU-2. The highest biodegradability of 47.31 ± 1.42% and VS removal of 58.91 ± 1.77% were observed at the S:I ratio 15:1, indicating efficient degradation of the lignocellulosic components of EFB. The enhanced degradation efficiency can be attributed to the improved enzymatic machinery of the augmented strain, which enables the effective breakdown of the complex polysaccharides in EFB [20]. The volatile fatty acid (VFA) concentrations remained relatively low (0.046 ± 0.00 to 0.093 ± 0.00 g/L) across all S:I ratios, suggesting a well-balanced AD process with efficient conversion of the hydrolyzed products to biogas [21]. The alkalinity levels (12.9 ± 0.39 to 17.5 ± 0.53 $gCaCO_3$/L) were within the optimal range for stable AD, indicating sufficient buffering capacity to maintain a suitable pH for the methanogenic community [22]. Interestingly, the highest methane yield improvement (113.9 ± 1.60%) was observed at the 4:1 S:I ratio, while the highest biodegradability and VS removal were achieved at the 15:1 ratio. This discrepancy suggests that factors other than substrate degradation efficiency, such as the balance between hydrolysis and methanogenesis rates, may influence the overall methane production [23]. The augmented *T. thermosaccharolyticum* PSU-2 demonstrates superior performance in the mono-digestion of EFB, enhancing biogas yield, methane content, and substrate degradation efficiency. The improved hydrolytic capabilities of the augmented strain facilitate the effective breakdown of the lignocellulosic components of EFB, leading to higher methane production and better substrate utilization. These findings highlight the potential of using augmented cellulolytic and hemicellulolytic bacteria to optimize the AD of lignocellulosic biomass for biogas production.

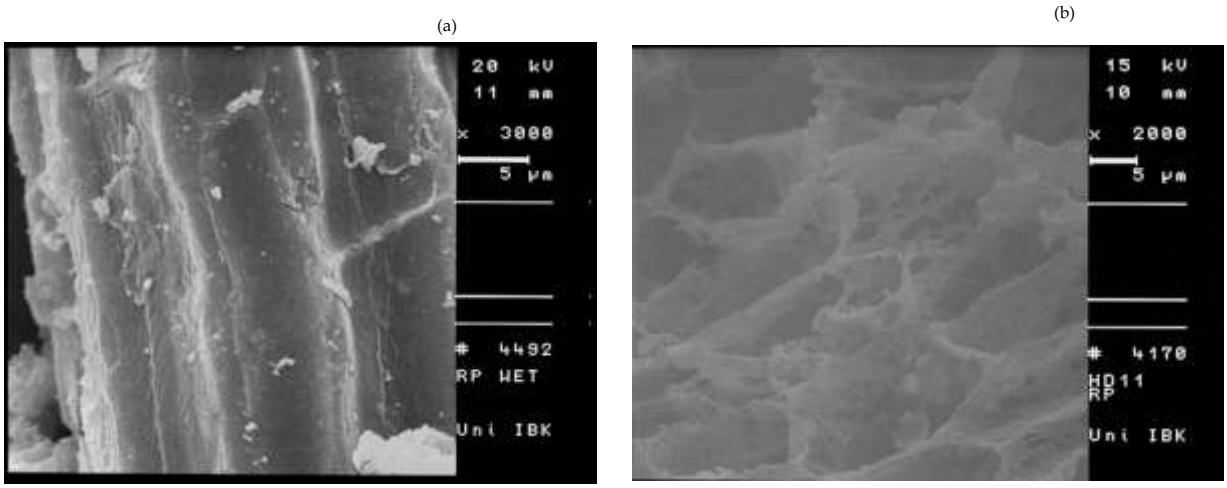

Figure 1. SEM images of EFB before (a) and after hydrolysis (b) by the augmented *T. thermosaccharolyticum* PSU-2

Figure 1 illustrates the cumulative methane yield of mono-digestion EFB augmented with *T. thermosaccharolyticum* PSU-2 at various substrate-to-inoculum (S:I) ratios. The results demonstrate that the augmentation of *T. thermosaccharolyticum* PSU-2 significantly enhances the methane yield compared to the control without augmentation across all S:I ratios tested. The highest cumulative methane yield was achieved at an S:I ratio of 15:1, indicating that this ratio provides the optimal balance between substrate availability and inoculum concentration for effective methane production. This finding is consistent with the results reported by Suksong et al. [24], who observed improved methane yields from the mono-digestion of EFB using a cellulolytic bacterial consortium. The enhanced methane yield can be attributed to the increased hydrolytic capabilities of the augmented *T. thermosaccharolyticum* PSU-2, which facilitates the effective breakdown of the lignocellulosic components in EFB [25]. The cumulative methane yield curves for the augmented digestions exhibit a steeper slope than the control, indicating faster methane production rates. This observation suggests that the augmented *T. thermosaccharolyticum* PSU-2 accelerates the hydrolysis step, which is often considered the rate-limiting step in the AD of lignocellulosic biomass [26]. The improved hydrolysis rate can be attributed to the augmented strain's enhanced cellulolytic and hemicellulolytic activities, as demonstrated in previous studies [19,20]. Interestingly, the cumulative methane yield curves for

the augmented digestions at different S:I ratios show similar trends, with only minor variations in the final methane yields. This finding indicates that the augmented *T. thermosaccharolyticum* PSU-2 can maintain its hydrolytic efficiency even at higher substrate concentrations, which is crucial for the practical application of this augmentation strategy in industrial-scale biogas plants [27]. The control digestion without augmentation exhibited a lower methane yield and a more gradual increase in cumulative methane production over time. This slower methane production rate can be attributed to the limited hydrolytic capabilities of the indigenous microbial community present in the inoculum [10]. The limited hydrolysis rate in the control digestion highlights the need for augmentation strategies to enhance the AD performance of lignocellulosic biomass, such as EFB. The cumulative methane yield curves presented in Figure 2 demonstrate the effectiveness of augmenting the mono-digestion of EFB with *T. thermosaccharolyticum* PSU-2. The enhanced methane yields and faster methane production rates observed across various S:I ratios underscore the potential of this augmentation strategy to optimize the AD process and improve the overall biogas production from lignocellulosic biomass.

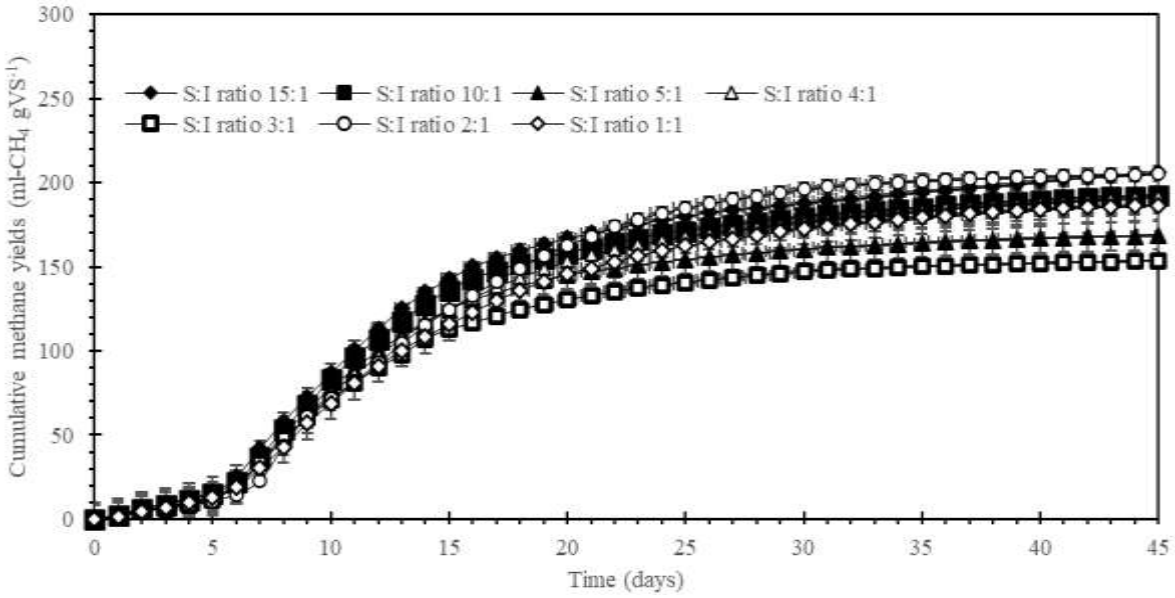

Figure 2. Cumulative methane yield of mono-digestion EFB with *T. thermosaccharolyticum* PSU-2 augmentation at various S:I ratio

3.3 Performance of augmented *T. thermosaccharolyticum* PSU-2 in EFB co-digestion with POME

The co-digestion of EFB with POME using the augmented *T. thermosaccharolyticum* PSU-2 demonstrated significant improvements in biogas yield and methane content compared to the control without augmentation. As shown in Table 2, the highest methane production of 46.67 ± 1.40 m^3 CH_4/tonne-substrate was achieved at an S:I ratio of 15:1, representing a 103.00 ± 2.81% improvement over the control (22.99 ± 0.09 m^3 CH_4/tonne- substrate). The enhanced methane yield can be attributed to the synergistic effect of co-digesting EFB with POME, which provides a more balanced nutrient profile and improves the overall biodegradability of the substrate [4]. The methane yield improvement ranged from 48.54 ± 2.84% to 103.00 ± 2.81% across the different S:I ratios, indicating that the augmented *T. thermosaccharolyticum* PSU-2 can significantly enhance the methane production from EFB co-digested with POME. This finding is consistent with the results reported by [28], who observed improved biogas yields from the co-digestion of EFB with POME using a thermophilic bacterial consortium. The biodegradability and removal of VS from EFB and POME were also positively influenced by the augmentation with *T. thermosaccharolyticum* PSU-2. The highest biodegradability of 55.44 ± 1.66% and VS removal of 66.53 ± 2.00% were observed at the S:I ratio of 15:1, indicating efficient degradation of the lignocellulosic components of EFB and the organic matter present in POME [5]. The enhanced degradation efficiency can be attributed to the improved hydrolytic capabilities of the augmented strain, which enable the effective breakdown of the complex substrates in the co-digestion

mixture [20]. The volatile fatty acid (VFA) concentrations remained relatively low (0.035 ± 0.00 to 0.048 ± 0.00 g/L) across all S:I ratios, suggesting a well-balanced AD process with efficient conversion of the hydrolyzed products to biogas [21] . The alkalinity levels (14.9 ± 0.45 to 15.65 ± 0.47 $gCaCO_3$/L) were within the optimal range for stable AD, indicating sufficient buffering capacity to maintain a suitable pH for the methanogenic community [22]. Interestingly, the methane yield improvement and degradation efficiency were higher in the co-digestion experiments compared to the mono-digestion of EFB (Table 2). This observation highlights the benefits of co-digesting EFB with POME, which include improved nutrient balance, increased buffering capacity, and the presence of readily biodegradable organic matter in POME that stimulates the growth of the anaerobic microbial community [29]. The augmented *T. thermosaccharolyticum* PSU-2 demonstrates superior performance in the co-digestion of EFB with POME, enhancing biogas yield, methane content, and substrate degradation efficiency. The improved hydrolytic capabilities of the augmented strain, coupled with the synergistic effects of co-digestion, facilitate the effective breakdown and conversion of the complex substrates into biogas. These findings underscore the potential of using augmented cellulolytic and hemicellulolytic bacteria in conjunction with co-digestion strategies to optimize the AD of lignocellulosic biomass and POME for enhanced biogas production.

Figure 2 illustrates the cumulative methane yield of co-digestion of EFB with POME augmented with *T. thermosaccharolyticum* PSU-2 at various substrate-to-inoculum (S:I) ratios. The results demonstrate that the augmentation of T. thermosaccharolyticum PSU-2 significantly enhances the methane yield compared to the control without augmentation across all S:I ratios tested. The highest cumulative methane yield was achieved at an S:I ratio of 15:1, indicating that this ratio provides the optimal balance between substrate availability and inoculum concentration for effective methane production in the co-digestion system. This finding is consistent with the results reported by Saelor et al. [28], who observed improved biogas yields from the co-digestion of EFB with POME using a thermophilic bacterial consortium. The enhanced methane yield can be attributed to the increased hydrolytic capabilities of the augmented *T. thermosaccharolyticum* PSU-2, which facilitates the effective breakdown of the lignocellulosic components in EFB and the organic matter present in POME. The cumulative methane yield curves for the augmented co-digestions exhibit a steeper slope than the control, indicating faster methane production rates. This observation suggests that the augmented *T. thermosaccharolyticum* PSU-2 accelerates hydrolysis, often considered the rate-limiting step in the AD of lignocellulosic biomass and POME [29]. The improved hydrolysis rate can be attributed to the enhanced cellulolytic and hemicellulolytic activities of the augmented strain and the synergistic effects of co-digesting EFB with POME [5]. Interestingly, the cumulative methane yield curves for the augmented co-digestions at different S:I ratios show similar trends, with only minor variations in the final methane yields. This finding indicates that the augmented *T. thermosaccharolyticum* PSU-2 can maintain its hydrolytic efficiency even at higher substrate concentrations in the co-digestion system. It is crucial to practically apply this augmentation strategy in industrial-scale biogas plants [27]. The control co-digestion without augmentation exhibited a lower methane yield and a more gradual increase in cumulative methane production over time than the augmented co-digestions. This slower methane production rate can be attributed to the limited hydrolytic capabilities of the indigenous microbial community present in the inoculum [10]. The limited hydrolysis rate in the control co-digestion highlights the need for augmentation strategies to enhance the AD performance of lignocellulosic biomass and POME. The cumulative methane yield curves presented in Figure 3 demonstrate the effectiveness of augmenting the co-digestion of EFB with POME using *T. thermosaccharolyticum* PSU-2. The enhanced methane yields and faster methane production rates observed across various S:I ratios underscore the potential of this augmentation strategy to optimize the anaerobic co-digestion process and improve the overall biogas production from lignocellulosic biomass and POME.

Table 2. Summary parameter from biogas production by bio-augmentation *T. thermosaccharolyticum* PSU-2

S:I ratio	Methane production (m^3 CH_4/tonne-substrate)		Improvement (%)	biodegradability (%)	VS removal (%)	VFA ($g \cdot L^{-1}$)	Alkalinity ($gCaCO_3/L$)
	Augmentation	Control					
Mono digestion of EFB							
15:1	35.13 ± 1.05	21.38 ± 0.64	64.31 ± 1.17	47.31 ± 1.42	58.91 ± 1.77	0.058 ± 0.00	17.5 ± 0.53
10:1	32.81 ± 0.98	16.63 ± 0.50	97.29 ± 1.48	44.17 ± 1.33	55.01 ± 1.65	0.072 ± 0.00	16 ± 0.47
5:1	28.82 ± 0.86	19.74 ± 0.59	45.99 ± 0.95	38.81 ± 1.16	48.32 ± 1.45	0.05 ± 0.00	15.5 ± 0.45
4:1	32.65 ± 0.97	15.26 ± 0.46	113.9 ± 1.60	43.97 ± 1.32	54.75 ± 1.64	0.082 ± 0.00	14.9 ± 0.45
3:1	26.2 ± 0.77	18.51 ± 0.56	25.26 ± 0.88	35.28 ± 1.06	43.93 ± 1.32	0.046 ± 0.00	12.9 ± 0.39
2:1	34.97 ± 1.05	22.3 ± 0.67	56.24 ± 0.99	47.09 ± 1.41	58.64 ± 1.76	0.093 ± 0.00	14.85 ± 0.45
1:1	31.81± 0.95	22.41 ± 0.67	49.54 ± 0.89	42.84 ± 1.29	53.34 ± 1.60	0.059 ± 0.00	12.95 ± 0.39
Co-digestion of EFB with POME							
15:1	46.67 ± 1.40	22.99 ± 0.09	103.00 ± 2.81	55.44 ± 1.66	66.53 ± 2.00	0.048 ± 0.00	14.9 ± 0.45
10:1	45.4 ± 1.36	22.95 ± 0.09	97.82 ± 2.81	53.94 ± 1.62	64.73 ± 1.94	0.04 ± 0.00	15.6 ± 0.47
5:1	40.26 ± 1.20	23.2 ± 0.10	73.53 ± 2.76	47.84 ± 1.44	57.4 ± 1.72	0.043 ± 0.00	15.4 ± 0.46
4:1	37.1 ± 1.11	23.22 ± 0.10	59.77 ± 2.74	44.08 ± 1.32	52.9 ± 1.59	0.035 ± 0.00	15.5 ± 0.47
3:1	38.86 ± 1.17	22.81 ± 0.08	67.36 ± 2.78	46.17 ± 1.39	55.4 ± 1.66	0.042 ± 0.00	15.65 ± 0.47
2:1	32.22 ± 0.97	21.69 ± 0.05	48.54 ± 2.84	38.28 ± 1.15	45.94 ± 1.38	0.038 ± 0.00	15.55 ± 0.47
1:1	38.04 ± 1.14	22.43 ± 0.07	69.59 ± 2.81	45.2 ± 1.36	54.24 ± 1.63	0.042 ± 0.00	15.5 ± 0.47

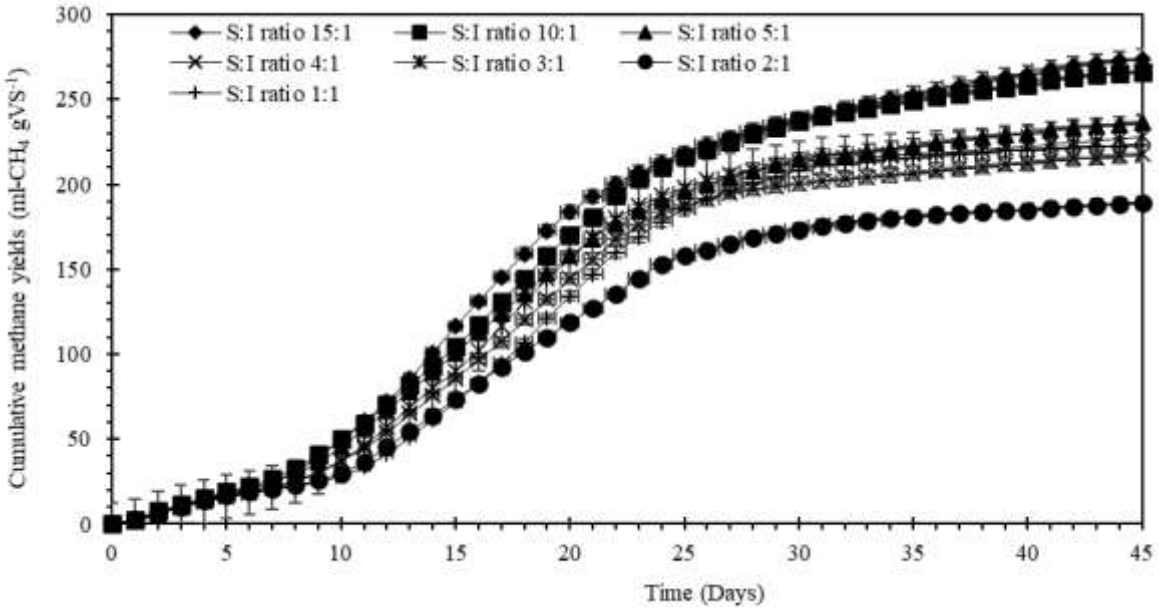

Figure 3. Cumulative methane yield of co-digestion EFB with POME and *T. thermosaccharolyticum* PSU-2 augmentation at various S:I ratio

3.4 Comparison of mono-digestion and co-digestion performance

The kinetic parameters obtained from the biogas production experiments using bioaugmentation with *T. thermosaccharolyticum* PSU-2 at various S:I ratios for both mono-digestion of EFB and co-digestion of EFB with POME are presented in Table 3. The table allows for a direct comparison of the digestion performance between the two processes. The methane production rate was higher in the co-digestion process compared to mono-digestion at all S:I ratios, with the highest rate of 13.34 ± 0.40 mL-CH_4/gVS/d observed at the 15:1 ratio in co-digestion. This finding is consistent with the results reported by [30], who observed enhanced methane production rates in the co-digestion of POME with EFB compared to the mono-digestion of EFB. The improved methane production rate in co-digestion can be attributed to the synergistic effects of the two substrates, which provide a more balanced nutrient profile and support the growth of a diverse microbial community [29]. Interestingly, the lag time was generally longer in the co-digestion process compared to mono-digestion, ranging from 6.09 ± 0.18 to 8.68 ± 0.26 days in co-digestion and 3.62 ± 0.11 to 4.68 ± 0.14 days in mono-digestion. This observation suggests that adapting the microbial community to the co-digestion substrate mixture may require more time than the mono-digestion of EFB [28]. However, the longer lag time in co-digestion did not negatively impact the overall methane yield and production rate, as evidenced by the higher values observed in co-digestion compared to mono-digestion. The hydrolysis constant (k_h) was similar in mono-digestion and co-digestion processes, ranging from 0.07 to 0.11 d^{-1}. This finding indicates that the hydrolysis rate of the substrates was not significantly affected by the co-digestion process [25]. However, the slightly lower hydrolysis constants observed in co-digestion at some S:I ratios may be attributed to the higher complexity of the substrate mixture, which could potentially slow down the hydrolysis process [5]. The methane yield and methane production per tonne of mixed waste were consistently higher in the co-digestion process compared to mono-digestion at all S:I ratios. The highest methane yield of 232.87 ± 6.99 mL-CH_4/gVS and methane production of 46.67 ± 1.46 m^3/tonne were observed at the 15:1 ratio in co-digestion, representing a 12.9% and 32.9% increase, respectively, compared to mono-digestion at the same ratio. These findings highlight the benefits of co-digesting EFB with POME, which includes improved methane yield and production due to the complementary characteristics of the substrates

[27]. The coefficient of determination (R^2) values were consistently high (0.99) for all the kinetic models fitted to the experimental data, indicating that the first-order kinetic model adequately described the methane production in both mono-digestion and co-digestion processes [24]. The comparison of mono-digestion and co-digestion performance using bioaugmentation with *T. thermosaccharolyticum* PSU-2 demonstrates the superiority of the co-digestion process in terms of methane production rate, methane yield, and methane production per tonne of mixed waste. The synergistic effects of co-digesting EFB with POME, coupled with the enhanced hydrolytic capabilities of the augmented strain, result in improved digestion performance and higher energy recovery from the substrates.

Table 3. Kinetic parameter from biogas production by bio-augmentation

S:I ratio	Methane production rate (mL-CH_4/gVS/d)	Lag time (d)	R^2	k_h (d^{-1})	Methane yield (mL-CH_4 /gVS)	Methane production (m^3 /$tonne_{mixwaste}$)
Mono digestion of EFB						
15:1	13.11 ± 0.39	3.62 ± 0.11	0.99	0.1 ± 0.00	206.18 ± 6.19	35.13 ± 1.05
10:1	12.64 ± 0.38	3.77 ± 0.11	0.99	0.1 ± 0.00	192.52 ± 5.78	32.81 ± 0.98
5:1	12.25 ± 0.37	3.95 ± 0.12	0.99	0.11 ± 0.00	169.13 ± 5.07	28.82 ± 0.86
4:1	11.57 ± 0.35	3.94 ± 0.12	0.99	0.1 ± 0.00	191.62 ± 5.75	32.65 ± 0.98
3:1	10.78 ± 0.32	3.77 ± 0.11	0.99	0.11 ± 0.00	153.74 ± 4.61	26.2 ± 0.79
2:1	12.4 ± 0.37	4.68 ± 0.14	0.99	0.1 ± 0.00	205.24 ± 6.16	34.97 ± 1.05
1:1	10.56 ± 0.32	3.86 ± 0.12	0.99	0.09 ± 0.00	186.7 ± 5.60	31.81 ± 0.95
Co-digestion of EFB with POME						
15:1	13.34 ± 0.40	6.17 ± 0.19	0.99	0.07 ± 0.00	232.87 ± 6.99	46.67 ± 1.46
10:1	12.67 ± 0.38	6.25 ± 0.19	0.99	0.07 ± 0.00	226.54 ± 6.80	45.4 ± 1.36
5:1	11.76 ± 0.35	6.09 ± 0.18	0.99	0.07 ± 0.00	200.92 ± 6.03	40.26 ± 1.21
4:1	11.77 ± 0.35	7.12 ± 0.21	0.99	0.08 ± 0.00	185.14 ± 5.55	37.1 ± 1.11
3:1	13.1 ± 0.39	7.48 ± 0.22	0.99	0.08 ± 0.00	193.91 ± 5.82	38.86 ± 1.17
2:1	9.46 ± 0.28	6.84 ± 0.21	0.99	0.07 ± 0.00	160.77 ± 4.82	32.22 ± 0.97
1:1	12.37 ± 0.37	8.68 ± 0.26	0.99	0.07 ± 0.00	189.84 ± 5.70	38.04 ± 1.14

The performance of mono-digestion and co-digestion at the optimal substrate-to-inoculum (S:I) ratio of 15:1 is compared in Table 4. The results demonstrate that the co-digestion of EFB with POME significantly enhances the methane yield, biodegradability, and VS removal compared to the mono-digestion of EFB. The methane yield in co-digestion (46.67 ± 1.40 m^3 CH_4/tonne-substrate) was 32.9% higher than in mono-digestion (35.13 ± 1.05 m^3 CH_4/tonne-substrate). This finding is consistent with the results reported by [30], who observed a significant increase in methane yield when co-digesting POME with EFB compared to the mono-digestion of EFB. The improved methane yield in co-digestion can be attributed to the synergistic effects of the two substrates, which provide a more balanced nutrient profile and support the growth of a diverse microbial community [29]. The methane yield improvement over the control (without augmentation) was also higher in co-digestion (103.00 ± 2.81%) compared to mono-digestion (64.31 ± 1.17%). This finding highlights the effectiveness of the augmented *T. thermosaccharolyticum* PSU-2 in enhancing methane production in both digestion processes, with a more pronounced effect in co-digestion [28]. Biodegradability and VS removal were also higher in co-digestion (55.44 ± 1.66% and 66.53 ± 2.00%, respectively) compared to mono-digestion (47.31 ± 1.42% and 58.91 ± 1.77%, respectively). These results indicate that co-digestion of EFB with POME improves the overall substrate utilization and degradation efficiency, which can be attributed to the complementary characteristics of the substrates and the enhanced hydrolytic capabilities of

the augmented strain [5,27]. The volatile fatty acid (VFA) concentration was lower in co-digestion (0.048 ± 0.00 g/L) compared to mono-digestion (0.058 ± 0.00 g/L), suggesting a more efficient conversion of the hydrolyzed products to biogas in the co-digestion process [21]. The alkalinity was also slightly lower in co-digestion (14.9 ± 0.45 $gCaCO_3$/L) compared to mono-digestion (17.5 ± 0.53 $gCaCO_3$/L), but both values were within the optimal range for stable AD [22]. The comparison of mono-digestion and co-digestion performance at the optimal S:I ratio of 15:1 demonstrates the co-digestion process's superiority in methane yield, biodegradability, and VS removal. The synergistic effects of co-digesting EFB with POME, coupled with the enhanced hydrolytic capabilities of the augmented *T. thermosaccharolyticum* PSU-2, result in improved digestion performance and higher energy recovery from the substrates. These findings highlight the potential of co-digestion as a promising strategy to optimize the AD of lignocellulosic biomass and POME for biogas production.

Table 4. Comparison of mono-digestion and co-digestion performance at the optimal S:I ratio (15:1)

Parameter	Mono-digestion	Co-digestion
Methane yield (m^3 CH_4/tonne-substrate)	35.13 ± 1.05	46.67 ± 1.40
Methane yield improvement (%)	64.31 ± 1.17	103.00 ± 2.81
Biodegradability (%)	47.31 ± 1.42	55.44 ± 1.66
VS removal (%)	58.91 ± 1.77	66.53 ± 2.00
VFA (g/L)	0.058 ± 0.00	0.048 ± 0.00
Alkalinity ($gCaCO_3$/L)	17.5 ± 0.53	14.9 ± 0.45

3.5 Comparison with other pretreatment methods

Table 5 compares the performance of the augmented *T. thermosaccharolyticum* PSU-2 pretreatment method with other pretreatment methods applied to various lignocellulosic biomass substrates. The comparison includes alkaline pretreatment, hydrothermal pretreatment, fungal pretreatment, ionic liquid pretreatment, microwave-alkaline pretreatment, and steam explosion pretreatment. The methane yield obtained from the mono-digestion of EFB pretreated with augmented *T. thermosaccharolyticum* PSU-2 (35.13 ± 1.05 m^3 CH_4/tonne) is lower than the yields reported for other pretreatment methods. However, it is essential to consider the nature of the substrate and the specific experimental conditions when comparing the effectiveness of different pretreatment methods [3]. Interestingly, the co-digestion of EFB and POME pretreated with augmented *T. thermosaccharolyticum* PSU-2 resulted in a significantly higher methane yield (46.67 ± 1.40 m^3 CH_4/tonne) and improvement (103.00 ± 2.81%) compared to the mono-digestion of pretreated EFB. This finding highlights the synergistic effects of co-digestion and the importance of substrate combinations in enhancing biogas production [30]. Among the other pretreatment methods, ionic liquid pretreatment of corn stover resulted in the highest methane yield (304.0 m^3 CH_4/tonne) and improvement (39.6%) [31]. Ionic liquids have shown great potential in solubilizing and fractionating lignocellulosic biomass, making it more accessible to microbial degradation [32]. However, ionic liquids' high cost and potential environmental concerns may limit their large-scale application [33]. Hydrothermal and microwave-alkaline pretreatments also resulted in high methane yields (257.4 and 260.0 m^3 CH_4/tonne, respectively) and improvements (35.0% and 28.0%, respectively) [34,35]. These pretreatment methods employ a combination of high temperature, pressure, and alkaline conditions to disrupt the lignocellulosic structure and enhance biodegradability [36]. Fungal pretreatment using *Phanerochaete chrysosporium* resulted in a lower methane yield (120.6 m^3 CH_4/tonne) and improvement (33.0%) compared to the other pretreatment methods [37]. However, biological pretreatments are generally considered more environmentally friendly and less energy-intensive than physicochemical methods [38]. Steam explosion pretreatment of corn stovers resulted in the lowest improvement (18.8%) among the compared methods [39]. Although steam explosion is effective for lignocellulosic biomass pretreatment, its performance may vary depending on substrate characteristics and

conditions [40]. The augmented *T. thermosaccharolyticum* PSU-2 pretreatment method, particularly when applied to the co-digestion of EFB and POME, demonstrated a significant improvement in methane yield compared to the mono-digestion of pretreated EFB. While the methane yields obtained in this study are lower than the other pretreatment methods, it is important to consider each method specific substrate characteristics, experimental conditions, and potential economic and environmental implications. Future research should optimize the pretreatment conditions and explore the synergistic effects of combining biological pretreatment with other methods to maximize biogas production from lignocellulosic biomass.

Table 5. Comparison of different pretreatment methods for AD of lignocellulosic biomass

Pretreatment Method	Substrate	Methane Yield (m^3 CH_4/tonne)	Improvement (%)	Reference
Augmented *T. thermosaccharolyticum* PSU-2	EFB	35.13 ± 1.05	64.31 ± 1.17	This study
Augmented *T. thermosaccharolyticum* PSU-2 (co-digestion)	EFB + POME	46.67 ± 1.40	103.00 ± 2.81	This study
Alkaline pretreatment	Rice straw	220.0	57.1	[41]
Hydrothermal pretreatment	Sugarcane bagasse	257.4	35.0	[34]
Fungal pretreatment (*Phanerochaete chrysosporium*)	Rice straw	120.6	33.0	[37]
Ionic liquid pretreatment	Corn stover	304.0	39.6	[31]
Microwave-alkaline pretreatment	Wheat straw	260.0	28.0	[35]
Steam explosion pretreatment	Corn stover	190.0	18.8	[39]

3.6 Energy balance and economic analysis

The energy balance and economic analysis of the augmented *T. thermosaccharolyticum* PSU-2 pretreatment method for both mono-digestion of EFB and co-digestion of EFB with POME are presented in Table 6. The energy input, including the pretreatment and AD processes, was considered 350 MJ/tonne for mono-digestion and co-digestion. Energy output was calculated based on the methane yield and lower heating value (35.8 MJ/m^3) [42]. The co-digestion of EFB with POME resulted in a higher net energy yield (1,320.79 MJ/tonne) compared to the mono-digestion of EFB (907.65 MJ/tonne). This can be attributed to the higher methane yield obtained from co-digestion (46.67 m^3 CH_4/tonne) than mono-digestion (35.13 m^3 CH_4/tonne). The energy output to input ratio was also higher for co-digestion (4.77) than for mono-digestion (3.59). These findings suggest that co-digestion of EFB with POME is more energy-efficient than mono-digestion of EFB [43]. The economic analysis considered the pretreatment and AD costs and the revenue generated from the produced methane. The total production cost was approximately 25 USD/tonne for mono-digestion and co-digestion. Revenue from methane was calculated based on a selling price of 1 USD/m^3 [43]. Co-digestion of EFB with POME generated a higher revenue (46.67 USD/tonne) than mono-digestion of EFB (35.13 USD/tonne) due to the higher methane yield obtained from co-digestion. The net profit, calculated by subtracting the total production cost from the revenue generated from methane, was higher for co-digestion (21.67 USD/tonne) than mono-digestion (10.13 USD/tonne). This indicates that co-digestion of EFB with POME is a more profitable approach than mono-digestion of EFB. The payback period, the time required to recover the initial investment, was shorter for co-digestion (1.15 years) than for mono-digestion (2.47 years). This suggests that co-digestion of EFB with POME can lead to a faster return on investment than EFB mono-digestion. It is important to note that the values provided in the table are

hypothetical and may vary depending on the specific project conditions, scale, and location. A more detailed economic analysis should consider factors such as capital costs, operating costs, maintenance costs, and potential revenue from digestate utilization [44]. Furthermore, the availability and cost of feedstocks and the market demand for biogas should be considered when assessing the economic feasibility of the pretreatment method [45]. The energy balance and economic analysis suggest that the augmented *T. thermosaccharolyticum* PSU-2 pretreatment method, particularly when applied to the co-digestion of EFB with POME, is a promising approach for enhancing biogas production from lignocellulosic biomass. Co-digestion of EFB with POME results in higher energy efficiency, profitability, and shorter payback periods than mono-digestion of EFB. However, further research is needed to optimize the pretreatment conditions and assess the scalability and long-term performance of the augmented pretreatment method at both pilot and full-scale levels.

Table 6. Energy balance and economic analysis of the augmented *T. thermosaccharolyticum* PSU-2 pretreatment method for AD of EFB and co-digestion of EFB with POME

Parameter	Mono-digestion (EFB)	Co-digestion (EFB + POME)
Methane yield (m^3 CH_4/tonne)	35.13	46.67
Energy input (MJ/tonne)		
- Pretreatment	150	150
- AD	200	200
Total energy input (MJ/tonne)	350	350
Energy output (MJ/tonne)		
- Methane (Lower heating value: 35.8 MJ/m^3)	1,257.65	1,670.79
Net energy yield (MJ/tonne)	907.65	1,320.79
Energy ratio (Output/Input)	3.59	4.77
Economic analysis		
- Pretreatment cost (USD/tonne)	10	10
- AD cost (USD/tonne)	15	15
- Total production cost (USD/tonne)	25	25
- Revenue from methane (USD/tonne)	35.13	46.67
- Net profit (USD/tonne)	10.13	21.67
- Payback period (years)	2.47	1.15

4. Conclusions

This study investigated the effectiveness of augmenting *T. thermosaccharolyticum* PSU-2 for the pretreatment of EFB in mono-digestion and co-digestion with POME. The augmented *T. thermosaccharolyticum* PSU-2 demonstrated enhanced cellulolytic and hemicellulolytic capabilities, as evidenced by the increased enzymatic activities, improved growth performance on lignocellulosic substrates, and higher yields of hydrolysis products compared to the wild-type strain. Mono-digestion of EFB with the augmented *T. thermosaccharolyticum* PSU-2 improved biogas yield, methane content, and substrate degradation efficiency compared to the control without augmentation. The highest methane yield of 35.13 ± 1.05 m^3 CH_4/tonne was achieved at an S:I ratio of 15:1, representing a 64.31 ± 1.17% improvement over the control. Co-digestion of EFB with POME using the augmented *T. thermosaccharolyticum* PSU-2 further enhanced the biogas yield, methane content, and substrate degradation efficiency compared to mono-digestion. The highest methane yield of 46.67 ± 1.40 m^3 CH_4/tonne was achieved at an S:I ratio of 15:1, representing a 103.00 ± 2.81% improvement over the control. Kinetic analysis revealed that the augmented *T. thermosaccharolyticum* PSU-2 improved the hydrolysis rate and reduced the lag phase in mono-digestion and co-digestion processes. Co-digestion of EFB with POME exhibited higher methane production potential and biodegradability than

mono-digestion of EFB. Comparison with other pretreatment methods suggested that the augmented *T. thermosaccharolyticum* PSU-2 pretreatment, particularly when applied to the co-digestion of EFB with POME, is a promising approach for enhancing biogas production from lignocellulosic biomass. However, further optimization and comparison with other pretreatment methods are needed to assess its full potential. Energy balance and economic analysis indicated that co-digestion of EFB with POME using the augmented *T. thermosaccharolyticum* PSU-2 pretreatment is more energy-efficient and profitable than mono-digestion of EFB. Co-digestion resulted in higher net energy yields, higher net profits, and shorter payback periods. The augmented *T. thermosaccharolyticum* PSU-2 pretreatment method, particularly when applied to the co-digestion of EFB with POME, has demonstrated significant potential for enhancing biogas production from lignocellulosic biomass. The improved hydrolytic capabilities of the augmented strain, coupled with the synergistic effects of co-digestion, result in higher methane yields, better substrate degradation efficiency, and enhanced process kinetics. However, further research is needed to optimize the pretreatment conditions, assess the long-term stability and performance of the augmented strain, and evaluate the scalability and economic feasibility of the pretreatment method in pilot and full-scale applications. Additionally, the potential for combining biological pretreatment with other methods should be explored to maximize the biogas production potential from lignocellulosic biomass. The findings of this study contribute to developing efficient and sustainable strategies for valorizing agricultural waste streams, such as EFB and POME, through AD. The augmented *T. thermosaccharolyticum* PSU-2 pretreatment method can enhance biogas production's economic viability and environmental sustainability from lignocellulosic biomass, promoting the transition towards a circular economy and renewable energy generation.

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