Research Article

Potential of Indigenous Microbes from Beef Cattle Faeces as Biological Agents in Pre-treatment of Bioethanol Production from Palm Oil Empty Fruit Bunches

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Abstract |Palm oil empty fruit bunches (EFB) contain high lignin levels that limit their conversion into fermentable sugars for bioethanol production. The integration of alkali pretreatment with indigenous microbial consortia derived from beef cattle faeces offers a sustainable and effective strategy to enhance lignocellulosic biomass degradation. This study evaluated the effect of alkaline pretreatment intensity on lignin removal, cellulose availability, glucose release during hydrolysis using indigenous microbes from beef cattle faeces, and subsequent changes in pH, C/N ratio, and ethanol production during fermentation. Hydrolysis was conducted using an indigenous microbial consortium from beef cattle faeces, while fermentation was carried out using *Saccharomyces cerevisiae*. Microbial composition was identified using 16S rRNA based next-generation sequencing (NGS). Lignin, cellulose, and glucose were analyzed using ANOVA with a 3×6 replication design, whereas fermentation parameters were evaluated using paired t-tests followed by regression analysis. The microbial community was dominated by Firmicutes, withe *Bacillus* as th dominant genus (±63%), supported by lignocellulolytic fungi (*Trichoderma, Rhizopus, and Mucor*). Alkali pretreatment modulated the biomass structure by significantly increasing cellulose availability (p<0,05), while lignin reduction and glucose yield showed no statiscally significant differences (p>0.05). Fermentation parameters showed significant changes ($p < 0.05$), including increased pH, decreased C/N ratio, and enhanced ethanol production. Regression analysis revealed a strong relationship between lignin and cellulose ($R^2 = 0.77$), while weaker relationships were observed between cellulose–glucose ($R^2 = 0.19$) and glucose–ethanol ($R^2 = 0.01$), indicating rapid sugar utilization during SSF. Ethanol production is governed by lignocellulosic accessibility and microbial activity rather than glucose accumulation, following a mechanistic SSF pathway of C/N reduction, glucose release, and ethanol formation.

Keywords: Bioethanol, Decomposition, Fermentation, Glucose, Lignocellulose, Microbial

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INTRODUCTION

Oil palm empty fruit bunches (EFB) and beef cattle faeces are abundant lignocellulosic wastes that pose environmental challenges if not managed properly. However, they offer significant potential as sustainable feedstocks for bioethanol production (Broda *et al.*, 2022; Sharker *et al.*, 2023; Mujtaba *et al.*, 2023; Yu B *et al.*, 2023). The balanced carbon and nitrogen (C/N) composition of these substrates plays a crucial role in regulating microbial

activity during biomass degradation, particularly the lignolytic and cellulolytic processes (Agustina *et al*., 2021).

However, the difficult-to-decompose structure of lignocellulose, where cellulose is tightly bound to lignin and hemicellulose, limits hydrolysis efficiency and requires effective pretreatment strategies (Zoghlami and Paës ., 2019; Pinto *et al*., 2022). Alkaline pretreatment is used to swell the biomass and partially disrupt lignin, while indigenous microbes produce enzymes that may further enhance the porosity and accessibility of the cellulose fibers. Similarly, indigenous microbes from beef cattle faeces, naturally adapted to degrade fibrous substrates in the rumen, produce lignolytic and cellulolytic enzymes that can accelerate biomass conversion (Wu *et al*., 2020a; Wu *et al*., 2023).

In a simultaneous Saccharification and fermentation (SSF) system, a reduction in the C/N ratio reflects enhanced microbial degradation, leading to increased glucose release during hydrolysis and subsequent higher ethanol production during fermentation (Santos *et al*., 2026). Therefore, integrating alkaline pretreatment with an indigenous microbial consortium is a promising, low-cost, and sustainable approach to improving lignocellulose bioconversion efficiency (Wu *et al*., 2020b; Abolore *et al*., 2024). Therefore, this study aimed to evaluate the potential of an indigenous microbial consortium from beef cattle faeces as a biological agent in EFB pretreatment integrated with alkaline pretreatment. Specifically, this study investigated the effects of pretreatment on lignin removal, cellulose accessibility, and glucose release during hydrolysis, as well as their subsequent impact on ethanol production in a simultaneous Saccharification and fermentation (SSF) system.

This study elucidates the metabolic flux and dynamic relationship between lignocellulose structural changes and bioconversion efficiency, emphasizing the rapid conversion of released glucose into ethanol within an SSF system. We hypothesized that the indigenous microbial consortium from cattle faeces acts in complement with alkali pretreatment to optimize lignocellulose degradation, thereby enhancing the mechanistic pathway of C/N reduction leading to glucose release and ethanol production in the SSF system.

MATERIALS AND METHODS

Materials

The materials used in this study consisted of EFB which had been dried and reduced in size using a chopper, fresh beef cattle faeces, obtained from the Faculty of Animal Husbandry's beef cattle pen, were used directly to ensure the stability of the indigenous microbial community for the decomposition process, by mixing the EFB with the beef cattle faeces until homogeneous. The ratio of EFB to beef cattle faeces used was 1:1. Decomposition was carried out for 7 days. Other material included NaOH, destilled water (aquades), and *Saccharomyces cerevisiae* as a starter used in the fermentation process. The research stages were as follows:

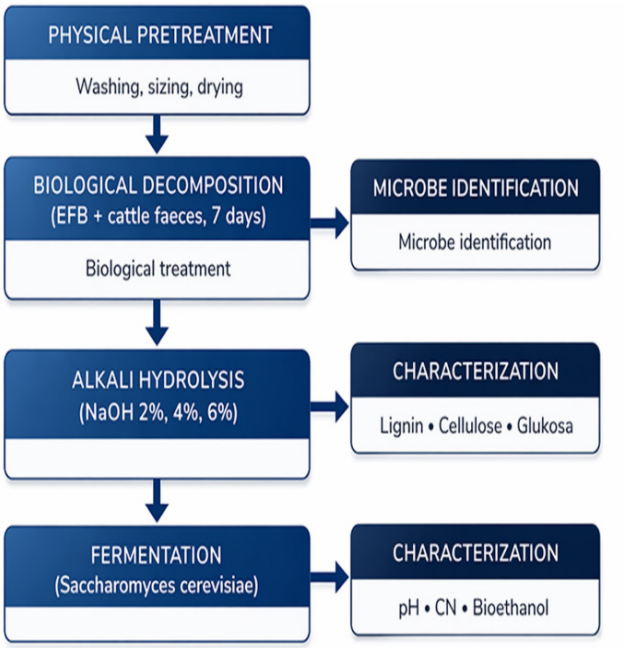

Figure 1: Flowchart of the research.

Microbe identification

Using next-generation sequencing (NGS) of 16S rRNA to identify the phylum and genus of bacteria found in beef cattle faeces, bacterial identification was performed using the Total Plate Count (TPC) method (Pakpour and Horgan, 2021) and fungal identification using spread plate method (Copetti *et al*., 2018). A 1-gram sample was placed in a test tube and homogenized with 9 ml of physiological NaCl to obtain dilutions ranging from 10^{-1} to 10^{-8}. The 10^{-4} dilution was inoculated into a Petri dish containing PDA, while the 10^{-8} dilution was inoculated into a Petri dish containing NA. The Petri dish containing NA was incubated for 24 hours at 37°C, while the Petri dish containing PDA was incubated for 5 x 24 hours at 25-30°C. The incubated bacterial and fungal colonies and microbial cultures were observed macroscopically and microscopically. Microscopic observations of characteristics, namely cell shape, cell arrangement, and Gram staining tests. For fungi or for molds, microscopic observations of characteristics, namely the shape of the spore head, asexual spores, and hyphae were carried out using the slide culture method (Chander, 2018).

Hydrolysis stage

Lignin and cellulose contents in the EFB mixture before and after hydrolysis were measured using the Chesson method with the following calculations:

Lignin content = (initial weight – final weight) / initial weight × 100%
Cellulose content = (final weight) / (initial weight of sample) × 100%

Measurement of glucose levels was carried out using a UV-Vis spectrophotometer with H_2SO_4 (sulfuric acid) reagent and a wavelength of 595-610 nm using the anthrone method. The standard solutions were aspirated one by one into the UV-Vis spectrophotometer and continued by aspirating the sample solution to analyze the absorbance value.

Fermentation stage

The Saccharomyces cerevisiae starter was prepared by adding 150 grams of granulated sugar to 1500 mL of distilled water in a sterile container. The mixture was sterilized by autoclaving at 121˚C for 15 minutes, then let cool to room temperature. Subsequently, 75 grams of commercial yeast was added and incubated for 48 hours at 30˚C. For the fermentation process, the entire hydrolysis mixture (including solid EFB residue and liquid fraction) was utilized to maintain the Simultaneous Saccharification and Fermentation (SSF) system. To ensure consistency across replicates, the mixture was thoroughly homogenized before transferring 1000 mL of the slurry into the fermentation vessel. Finally, 10% (v/v) of the starter volume was added, and fermentation was carried out for 7 days.

pH, Carbon, and Nitrogent Content; pH measurements were performed using a pH meter, using buffer solutions of pH 4 and 7; Carbon content was measured using the Walkley-Black method, which uses the principle that organic carbon is oxidized by dichromate in an acidic environment. Nitrogent content was determined using the Kjeldahl method.

Bioethanol

Bioethanol content was measured using a UV-Vis spectrophotometer with potassium dichromate ($K_2Cr_2O_7$) as the reagent and a wavelength of 595-610 nm. Standard solutions were aspirated one by one into the UV-Vis spectrophotometer, followed by aspirating the sample solution for absorbance analysis.

Design experiment

The research was conducted using an experimental method based on a Completely Randomized Design (CRD). For the hydrolysis stage, the variables measured were lignin, cellulose, and glucose. The treatments consisted of three levels of NaOH concentration: P1=2%, P2=4%, P3= 6%, with six replications each. The focus of this study was to evaluate the optimization of alkali concentration in conjunction with a fixed dose of indigenous microbial consortia.

For the fermentation stage (pH, Carbon, and Bioethanol), the same treatment groups were monitored. Data were analyzed using paired sample t-tests to evaluate the significant changes before and after fermentation within each treatment. To compare the effectiveness between different NaOH concentrations, a one-way Analysis of Variance (ANOVA) followed by Duncan's Multiple Range Test was performed. Regression analysis was subsequently utilized to determine the relationships between chemical compositions, environmental parameters, and final ethanol yield.

RESULTS AND DISCUSSION

Ecological diversity of microbial consortia from beef cattle faeces based on genus

Analysis of microbial ecological diversity was conducted to identify the bacteria involved in the hydrolysis pretreatment process. The analysis was performed using Next Generation Sequencing (NGS) technology with 16S rRNA primers, are shown in Figure 2.

In Figure 2 it can be seen that the phylum is dominated by Firmicutes. The Firmicutes phylum plays an important role in the biomass hydrolysis stage and fermentation

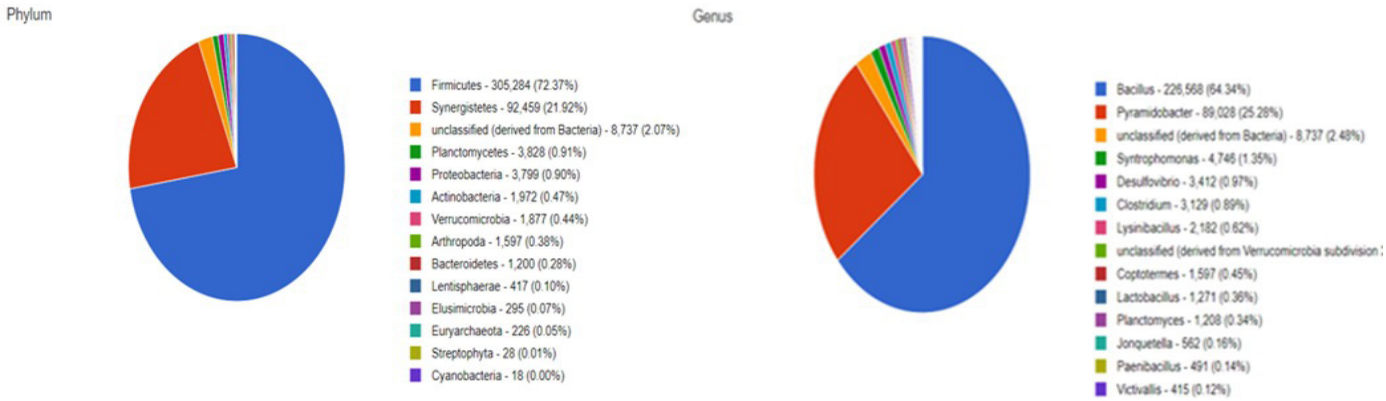

Figure 2: Types and proportions of bacteria in the biological process.

process. Firmicutes are important in degradation because they use free enzymes and cellulosomes to degrade EFB polysaccharides. (Gharechahi *et al*., 2023). Figure 2 shows that microbial diversity at the genus level is dominated by Bacillus (64.34%), followed by Pyramidobacter 25.26%. Other genera included unclassified bacteria (2.48%), Syntrophomonas (1.35%), and Clostridium (0.89%). Pyramidobacter is an anaerobic genus typically found in the rumen that excels in protein degradation and amino acid fermentation. Its presence, along with Clostridium 0.89%, suggests that despite the process being conducted in open containers, the dense structure of the EFB and faeces mixture created anaerobic micro-environments (niches) within the substrate. The dominance of Bacillus is attributed to its facultative anaerobic nature and its ability to form resilient spores, allowing it to survive the transition from the rumen to the pretreatment environment. This is consistent with studies showing that enzymatic hydrolysis and fermentation convert lignocellulosic sugars into bioethanol. Several bacterial species involved include *Clostridium spp, Bacillus spp,* and fungal species, for example, *Trichoderma spp*, which have been widely known in the biological pretreatment of lignocellulosic biomass (Sharma *et al*., 2019), Figures 2 is supported by the results of macroscopic characterization for the number of bacteria and fungi (Figures 3 and 4), and microscopic with Gram Staining. Most bacteria in this phase are indigenous bacteria from beef cattle faeces and EFB. In beef cattle, microbes such as bacteria generally develop in the rumen which will also be excreted with the faeces.

Microscopic morphology of indigenous bacteria and fungal isolates from beef cattle faeces

The common bacterial phylum in beef cattle fecal microbiota is Firmicutes (Zhang *et al*., 2021). The most famous genus that is often used as a fiber degrader from the Firmicutes phylum is Bacillus (Hashmi *et al*., 2020). Both the phylum and genus are groups that play a good role in degrading fiber in the form of cellulose, hemicellulose, and lignocellulose. In EFB there are also indigenous cellulolytic bacteria.

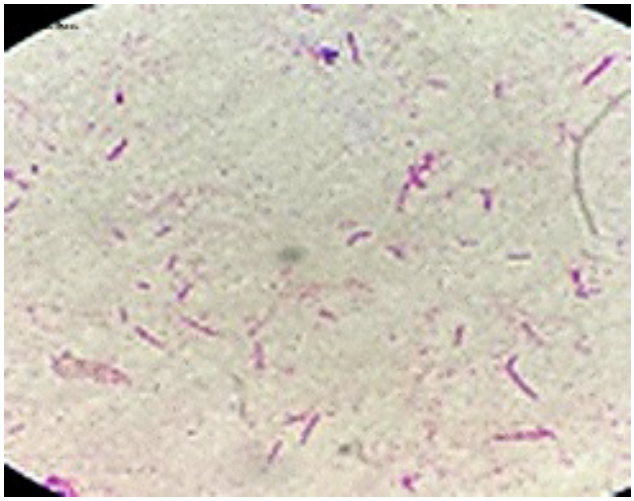

Figure 3: Bacillus.

Three genera of molds matched the characteristics and microscopic observations of samples from the biological pre-treatment of a mixture of beef cattle faeces and EFB. The genera obtained are *Rhizopus, Trichoderma*, and *Mucor*. The synergistic activity of Bacillus and filamentous fungi (*Trichoderma, Rhizopus, and Mucor*) significantly enhanced lignocellulose deconstruction, where partial delignification by bacteria increased cellulose accessibility, followed by efficient enzymatic hydrolysis to glucose by fungal cellulases. (Yan *et al*., 2018; Harlia *et al*., 2023; Kesumaningwati *et al*., 2024). The biological pretreatment was further supported by the presence of three indigenous fungal genera: *Rhizopus* sp, *Trichoderma* sp, and *Mucor* (Figures 4A-C). These filamentous fungi are well-documented for their robust production of cellulolytic and hemicellulolytic enzymes, such as endoglucanases and beta-glucosidases. In this integrated system, these fungi work in tandem with the bacterial consortium. While *Bacillus* initiates the deconstruction by increasing the porosity of the lignocellulosic matrix, the fungal secretome further hydrolyzes the exposed cellulose fibers into fermentable glucose. The dominance of *Trichoderma* sp. is particularly significant, as it is known to produce a complete set of cellulases that effectively overcome the recalcitrance of Palm Oil Empty Fruit Bunches (EFB).

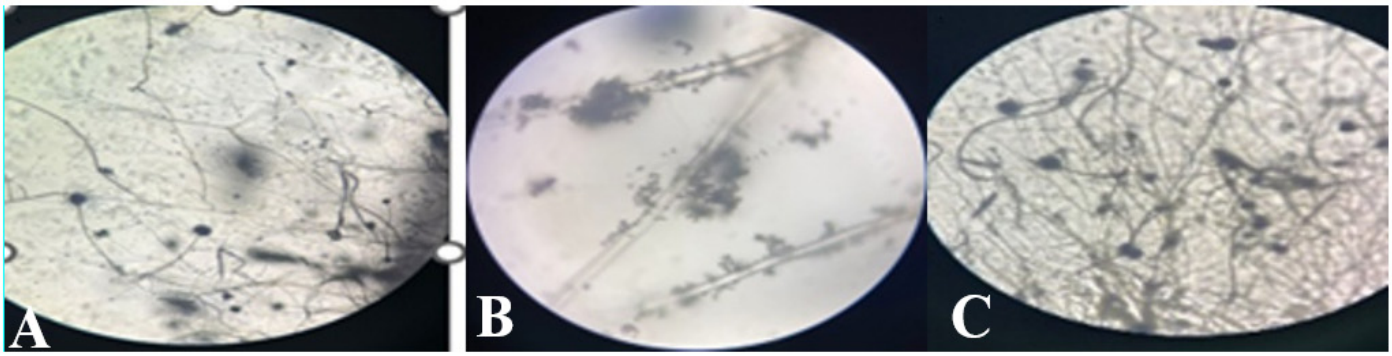

Figure 4: A: *Rhizopus* sp., **B:** *Trichoderma* sp., **C:** Mucor.

Effect of treatment on lignin, cellulosa and glucose levels in hydrolysis stage

The effects of alkali pretreatment on the lignocellulosic components are summarized in Table 1. In this study, the cellulose content significantly increased to 3.01% under treatment P1 ($p < 0.05$), indicating successful biomass deconstruction and increased fiber accessibility. In contrast, treatment P3 resulted in a lower cellulose percentage of 0.85%. While inhibitory compounds were not specifically analyzed, the reduction in both cellulose and glucose levels in P3 suggests that excessive pretreatment intensity did not further enhance the availability of fermentable sugars, potentially due to the degradation of the polysaccharide matrix.

Table 1: Numerical Values of Lignin and Cellulose Post-Pretreatment

Treatment	Lignin (%)	Celullose (%)	Glucose (%)
P1	0,42±0,20a	3,01±1,17b	0,030±0,023a
P2	0,48±0,20a	2,09±1,17ab	0,038±0,023a
P3	0,21±0,20a	0,85±1,17a	0,027±0,023a
P-value	0,12	0,039*	0,753

Hydrolysis is carried out to separate and reduce lignin bonds with hemicellulose and break down the cellulose structure into simple sugars (Figure 5).

Figure 5 alkaline pretreatment significantly reduced lignin content and modified cellulose structure ($p < 0.05$), confirming its effectiveness in improving biomass accessibility. The decrease in cellulose availability and glucose yield observed in P3 suggests that high-intensity alkaline pretreatment may lead to biomass degradation or structural changes that hinder enzymatic accessibility, rather than improving it. In contrast, moderate pretreatment (P2) produced the highest glucose yield, indicating an optimal balance between effective delignification and preservation of the cellulose matrix. Pretreatment is considered successful if the cellulose percentage increases. An increase in the cellulose percentage indicates that cellulose can be easily accessed during the hydrolysis process (khairiah and Ridwan., 2021). These findings are consistent with previous reports that appropriate pretreatment enhances enzyme accessibility without degradation of fermentable sugars (Sharma *et al*., 2019; Wu *et al*., 2020a; Zhang *et al*., 2021).

Fungal genera such as *Trichoderma*, *Rhizopus*, and *Mucor* play a critical role in lignocellulose degradation through the production of cellulolytic enzymes that convert cellulose into glucose. Meanwhile, bacterial genera including *Bacillus* and *Clostridium* contribute synergistically to biomass degradation and fermentation via enzymatic activity and metabolic processes (Shukla *et al*., 2023). The significantly higher glucose yield observed in P2 suggests that optimal hydrolysis was achieved through the interaction between chemical pretreatment and microbial consortia.

Effect of treatment on pH at the fermentation stage

NaOH pretreatment of 2-6% in EFB decompost and beef cattle faeces showed a change in pH after the fermentation process. Detailed of impact is presented in Table 2.

Table 2: T test results of the effect of treatment on pH.

Treatment	Initial(Mean±SD)	Final(Mean±SD)	P value sign
P1	5.92±0.13	6.18±0.13	0.0029 *
P2	5.96±0.32	6.22±0.31	0.0070 *
P3	5.94±0.11	6.30±0.10	0.0086 *

Note: **= significant difference ($p < 0.05$) between the initial and final treatment.

The pH in all treatments increased by the 7th day (Table 2). This increase is not caused by ethanol production, as ethanol is a neutral molecules (C_2H_5OH) that does not dissociate inti hydroxyl ions (OH^-). Instead, the pH rise is attributed to proteolysis (protein degradation) of the organic matter from beef cattle faeces by the microbial consortium, which releases ammonia (NH_3). Ammonia acts as a weak base in the substrate, thereby increasing the pH (Zhang *et al*., 2021).

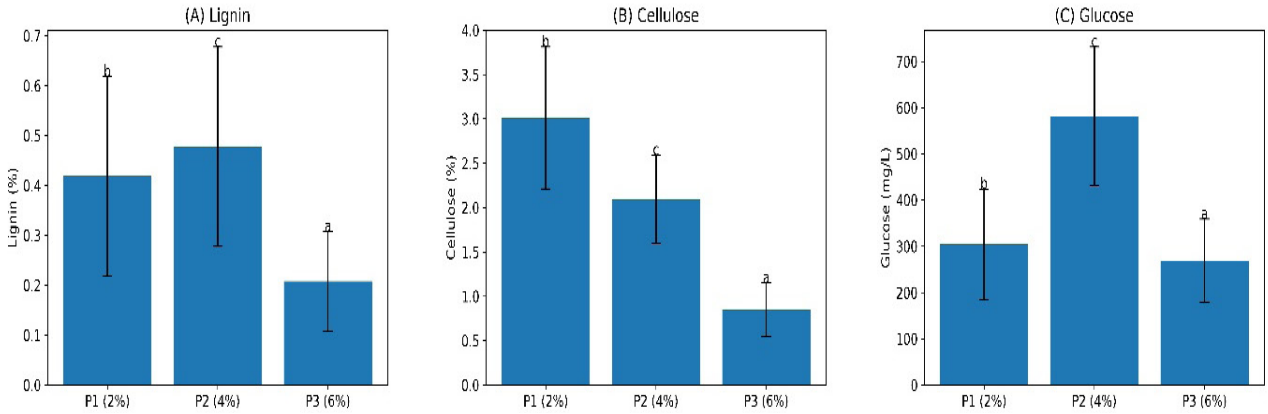

Figure5: Effect of treatment on lignin, cellulose and glucose levels.

While yeast growth typically produces CO_2 and organic acids that would normally lower the pH, in this SSF system, the buffering capacity of the ammonia released from the nitrogen-rich faeces dominates the pH profile. Furthermore, the use of NaOH during pretreatment contributes to the initial alkaline conditions. The degradation of protein into basic nitrogenous compounds by the consortium during fermentation explains the net increase in pH observed at the final stage, providing a suitable environment for the integrated activity of bacteria and yeast (pH 6.1–6.3).

The higher the NaOH concentration added during pretreatment, the substrate pH increases. This is because NaOH, a caustic alkali, is capable of releasing heat when dissolved in water, resulting in the formation of a strong base. Giving NaOH pretreatment is able to produce more sugar which results in the resulting ethanol also tending to be higher (Shen *et al*., 2023).

Effect of treatment on the carbon-to-nitrogen (C/N) ratio

Carbon in the fermentation process is needed as an energy source for microorganisms. The Carbon levels before and after fermentation are shown in Table 3.

Table 3: Effect of treatments on the carbon-to-nitrogen (C/N) ratio.

Parameter	Treatment	Initial (Mean±SD)	Final (Mean±SD)	p value	Sign
C/N ratio	P1	58.29±30.40	26.37±20.67	0.196	ns
	P2	79.48±36.62	53.14±27.73	0.019	*
	P3	108.57±103.66	81.42±106.03	0.050	*

ns = not significant ($p > 0.05$); *= significant difference ($p < 0.05$)

As shown in Table 2 a significant decline in the C/N ratio was observed in treatments P2 ($p = 0.019$) and P3 ($p = 0.050$). The high standard deviations observed, particularly in the initial stages, reflect the inherent heterogeneity of the solid-state substrate consisting of Oil Palm EFB and fresh faeces. Despite this variability, the consistent reduction in the C/N ratio indicates an active bioconversion process.

The decrease in the C/N ratio is primarily driven by the reduction in Total Organic Carbon (TOC) due to microbial mineralization. During fermentation, the microbial consortium utilizes various carbonaceous fractions—including cellulose, hemicellulose, and soluble sugars—for energy and cell synthesis. A significant portion of this carbon is lost from the substrate via microbial respiration as CO_2 (Aresta, 2022). While the conversion of glucose into bioethanol contributes to the carbon flux, the overall reduction in TOC is influenced by the collective degradation of the organic matrix. Since nitrogen levels remain relatively constant or are concentrated by the loss of carbon, the resulting C/N ratio decreases, confirming the stability and progression of the SSF system (Tse *et al*., 2021).

Effect of treatment on bioethanol

The ethanol production levels during the 7-day SSF process are presented in Table 4.

Based on the results in Table 4 all treatments experienced a numerical increase in ethanol levels by the end of the fermentation period. Analysis using one-way ANOVA showed that there were no significant differences in the final ethanol yields between P1, P2, and P3 ($p = 0.485$). This suggests that within the range of 2% to 6%, the NaOH concentration did not statistically differentiate the final ethanol output. However, the paired t-test confirmed that only treatment P2 (4% NaOH) achieved a statistically significant increase from the initial state ($p = 0.028$), indicating a more consistent bioconversion process at this concentration.

Table 4: Ethanol concentration during the Simultaneous Saccharification and Fermentation (SSF).

Treatment	Initial Ethanol (%)	Final ethanol (%)	p-value (t-test)	Anova Notations
P1	2,87 ± 0,93	3,23 ± 0,89	0,235	3,23[a]
P2	2,93 ± 0,76	3,67 ± 1,20	0,028*	3,67[a]
P3	3,47 ± 1,21	4,14 ± 1,26	0.065	4,14[a]

Note: * indicates a significant increase from initial to final fermentation within the treatment ($p < 0.05$). Superscript 'a' indicates no significant difference between treatments ($p = 0.485$) based on one-way ANOVA.

The higher the NaOH concentration used in the pretreatment process, the more effectively lignin can be degraded, allowing for higher cellulose conversion into fermentable sugars. Sodium hydroxide facilitates the separation of lignin from fiber by entering the Oil Palm Empty Fruit Bunches (EFB) and breaking down the complex lignin structure. Lignin dissolves at high pH because its phenolic hydroxyl groups are ionized to form polar salts, thereby increasing the accessibility of cellulose to microbial enzymes (Melro *et al*., 2020; Shah *et al*., 2023).

During the SSF process, the indigenous microbial consortium and *Saccharomyces cerevisiae* work synergistically to convert these mobilized carbohydrates into ethanol. The metabolic efficiency of this process is highly dependent on the environmental pH. Generally, yeast is an acidophilic organism that thrives in environments with an optimal pH range of 3.0 to 6.0 (Polprasert *et al*., 2021).

In this study, the final pH (6.1–6.3) remained conducive to

microbial activity. Maintaining a stable extracellular pH is

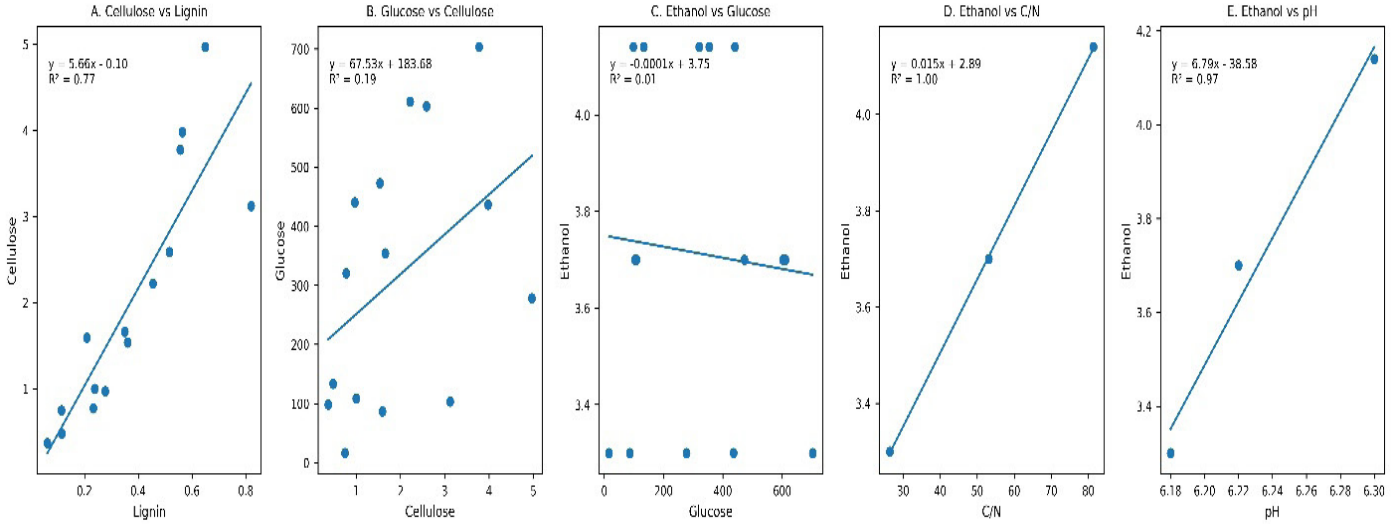

Figure 6: The relationships between (A) cellulose and lignin, (B) glucose and cellulose, (C) ethanol and glucose, (D) ethanol and C/N ratio, and (E) ethanol and pH.

critical, if the pH deviates too far from the optimal range, yeast cells struggle to maintain intracellular homeostasis, which can lead to enzyme deactivation. If enzymes are deactivated, the cells cannot reproduce or produce ethanol efficiently (Salihu *et al*., 2022). The successful increase in ethanol concentration, particularly the consistent trend in P2, confirms that the system provided a suitable environment for the integrated metabolic pathways of the consortium.

Simultaneous saccharification and fermentation (SSF)

Lignocellulosic composition, nutrient balance, and fermentation conditions influence the efficiency of hydrolysis and ethanol formation within the SSF system, as shown Figure 6.

The regression analysis in Figure 6 illustrates the complex dynamics of the SSF process. The strong correlation between lignin anda cellulose (R^2 = 0,77) indicates that lignin limits cellulose accessibility by acting as a structural barrier (Xie and Fan, 2025).

However, the weak relationship between cellulose and glucose (R^2 = 0,19) and the nearly non-existent correlation between residual glucose and ethanol (R^2= 0.01) are characteristic indicators of a high-efficiency SSF system. In such systems, glucose does not accumulate because it is converted into ethanol by *Saccharomyces cerevisiae* as quickly as it is released from the cellulose matrix. This rapid flux prevents the metabolic 'bottleneck' typically seen in separate hydrolysis processes, thus explaining why static measurements of glucose do not linearly predict ethanol yield (Weimer, 2022).

In contrast, the exceptionally strong correlation between ethanol yield and pH stability (R^2 = 0,97) highlights that environmental regulation is the primary driver of microbial performance in this study. The observed pH range (6.1–6.3) provided the optimal window for the synergistic action of the consortium, where even slight deviations in pH significantly impacted the metabolic carbon flux toward ethanol (Liu *et al*., 2025).

CONCLUSION

This study demonstrates that the integrated use of alkaline pretreatment and an indigenous microbial consortium derived from beef cattle faeces facilitates the bioconversion of Oil Palm Empty Fruit Bunches (EFB) into bioethanol. The microbial community, characterized by the presence of *Firmicutes* (notably *Bacillus*) alongside lignocellulolytic fungi (*Rhizopus*, *Trichoderma*, and *Mucor*), was associated with the degradation of the lignocellulosic matrix and subsequent ethanol formation under Simultaneous Saccharification and Fermentation (SSF) conditions.

Alkaline pretreatment effectively reduced lignin and cellulose levels, which increased the structural accessibility of the biomass. Statistical analysis confirmed that while ethanol yield did not significantly differ across the tested NaOH concentrations (2–6%), the process was characterized by a significant reduction in the C/N ratio and stable pH conditions. Regression analysis further highlights the efficiency of the SSF system, where ethanol production is primarily governed by lignocellulosic accessibility and the rapid microbial conversion of released sugars rather than glucose accumulation. These findings suggest that utilizing indigenous consortia for the treatment of agricultural waste offers a functional pathway

for lignocellulosic bioethanol production, provided that environmental parameters such as pH and C/N balance are strictly regulated.

ACKNOWLEDGEMENTS

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NOVELTY STATEMENT

The novelty of this manuscript in demonstrating that ethanol production is not directly governed by glucose accumulation but by the synchronization of saccharification and fermentation driven by microbial activity. To our knowledge, this is the first report that combines microbial ecology, path analysis, and SSF modelling to explain bioethanol formation from EFB using beef cattle faeces as an inoculum.

AUTHOR'S CONTRIBUTION

Ellin H. conceived and designed the study Alliyatul and Millenia. Conducted the experiments and collected the data. Ellin H. Yuli A. and Eulis T.M. Supervised the research and validated the methodology. Gilang L. U.S. Contributed to technical analysis and bioethanol process evaluation. Norli I. contributed to methodology development, result interpretation, and manuscript revision. Ellinn H. drafted the manuscript. All authors reviewed, revised and approved the final manuscript.

Generative AI and AI assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Conflict of interest

The authors declare no conflict of interest regarding this manuscript.

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