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OBTAINING BIOETHANOL FROM COCOA SHELLS (*THEOBROMA CACAO*) USING *TRICHODERMA REESEI* AND *TRICHODERMA GHANENSE* FOR ENZYMATIC HYDROLYSIS

OBTENCIÓN DE BIOETANOL A PARTIR DE LA CÁSCARA DE CACAO (*THEOBROMA CACAO*) USANDO *TRICHODERMA REESEI* Y *TRICHODERMA GHANENSE* PARA LA HIDRÓLISIS ENZIMÁTICA

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Abstract

The use of fossil fuels generates Greenhouse Gases (GHG), one of the main causes of global overheating, which has become a problem in recent decades. The use of second generation of biofuels has been perceived as an alternative to replace or reduce the use of fossil fuels; for this reason, the present work aims to obtain bioethanol from cocoa shell (*Theobroma cacao*) of the clone CCN-51 obtained in Los Rios Province, Ecuador, through a series of steps involving: a) alkaline pretreatment, b) enzymatic hydrolysis using two species of endophytic fungi from the same cocoa shell (*Trichoderma reesei* and *Trichoderma ghanense*) at different concentration and c) alcoholic fermentation using *Saccharomyces cerevisiae* yeast. The amount of bioethanol obtained from the process was determined by gas chromatograph with a flame ionization detector (FID). the results show a moderate production of bioethanol ranging from 0.024% v/v to

0.254% v/v, which indicates that the cocoa shell (*Theobroma cacao*) of clone CCN-51 is a potential matrix to bioethanol production.

Keywords: *Theobroma cacao*, *Trichoderma*, biomass, bioethanol, alcoholic fermentation.

Resumen

El uso de combustibles fósiles genera gases de efecto invernadero (GEI), uno de los principales causantes del sobrecalentamiento global, problemática de gran interés en las últimas décadas. El uso de biocombustibles de segunda generación se ha vislumbrado como alternativa para sustituir o disminuir el uso de combustibles fósiles. Por esta razón, el presente trabajo tiene como objetivo obtener bioetanol a partir de la cáscara de cacao (*Theobroma cacao*) del clon CCN-51 obtenido en la Provincia de Los Ríos, Ecuador, por medio de una serie de pasos que involucran: a) pretratamiento alcalino, b) hidrólisis enzimática usando dos especies de hongos endófitos de la misma cáscara de cacao (*Trichoderma reesei* y *Trichoderma ghanense*) a diferentes concentraciones y c) fermentación alcohólica usando levadura *Saccharomyces cerevisiae*. La cantidad de bioetanol obtenida del proceso fue determinada por medio de un cromatógrafo de gases con detector de ionización de llama (FID). Los resultados muestran una producción moderada de bioetanol que va desde 0,024% v/v a 0,254% v/v lo que indica que la cáscara de cacao (*Theobroma cacao*) del clon CCN-51 es una matriz potencial para la producción de bioetanol.

Palabras clave: *Theobroma cacao*, *Trichoderma*, biomasa, bioetanol, fermentación alcohólica.

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1 Introduction

Over time, human activity and industrial development have positioned fossil fuels as the primary energy source. Throughout their extraction and production processes, a significant number of countries, including Ecuador, have experienced economic benefits. However, these processes have also triggered various problems that extend beyond the producing countries and impact the global community. One notable example is the emission of greenhouse gases (GHGs), which are largely responsible for global warming.

In recent decades, scientific research has increasingly focused on mitigating these environmental impacts, leading to a growing interest in second-generation biofuels (Wahono et al., 2014; Oliva et al., 2017). This interest is largely due to the use of lignocellulosic biomass in their production-biomass that can be sourced from a variety of materials, such as agro-industrial residues from sugarcane and maize cultivation (Antizar-Ladislao and Turrion-Gomez, 2008). This enables the valorization of plant material that is typically discarded (Khan et al., 2025), resulting in a dual environmental benefit: first, the avoidance of waste accumulation on land surfaces and the need for its disposal through incomplete combustion, which is even more polluting (Cury R et al., 2017; Orejuela-Escobar et al., 2021); and second, the use of these second-generation fuels significantly reduces the proportion of GHGs released into the environment (Morais et al., 2020).

Preliminary studies suggest that residues from agricultural crops (such as stalks, leaves, and husks), non-food crops, forest residues, and agro-industrial waste could potentially support the required bioethanol supply (Anwar et al., 2014). Ecuador, as a country rich in plant diversity, has the capacity to generate lignocellulosic material suitable for this purpose.

According to records from 2007 to 2012, Ecuador experienced a 184% increase in the export of roasted cocoa beans and cocoa husks, positioning the country as one of the world's leading cocoa producers and exporters, reaching the fourth place globally (Teneda Llerena et al., 2019). Within the chocolate industry (Caviedes Rubio et al., 2024), only the cocoa beans-constituting approximately 30% of the entire fruit-are utilized in production, while the remaining 70% (husks and mucilaginous pulp) is discarded (Sarmiento Hernández, 2019). Considering the substantial amount of waste generated annually in Ecuador from this plant species, it has been estimated that bioethanol production from such biomass could serve as an alternative pathway for energy generation, significantly contributing to national fuel consumption (Sigüencia Avila et al., 2020). However, this potential has yet to be realized.

Moreover, it is important to note that the composition of lignocellulosic material varies depending on its origin (Anwar et al., 2014), and cocoa husks are no exception. They are reported to have a low cellulose content that fluctuates depending on the type of cocoa, along with a high proportion of lignin and hemicellulose-components that interfere with the conversion of cellulose into biofuel (Sarmiento Hernández, 2019). Combined with a general lack of information and awareness on the subject, these factors constitute the primary reasons why agro-industrial residues are not being properly utilized.

The chemical and enzymatic reactions necessary for bioethanol production are directly affected by the composition of lignocellulosic material (Winarsih and Siskawardani, 2020). In light of this and the challenges mentioned above, it is essential to develop alternative approaches that enable the use of biomass with conversion rates that make the process economically viable.

Accordingly, lignocellulosic material must undergo pretreatment followed by enzymatic hydrolysis-a method proven to be effective, cost-efficient, and specific for obtaining fermentable sugars under mild reaction conditions (Winarsih and Siskawardani, 2020). The efficiency of this process depends on several factors including pH, fermentation time, substrate type (biomass), temperature, and enzymatic activity, among others (Anwar et al., 2014). Numerous microorganisms are capable of degrading cellulose, among which fungi of the genus *Trichoderma* are the most common (Nasir Iqbal et al., 2011; Rosyida et al., 2015). In particular, the species *Trichoderma reesei* is the most commercially used due to its widespread industrial application in the saccharification of cellulose into simple sugars for biofuel production (Adav et al., 2012; Peciulyte et al., 2014).

This study aims to explore the necessary conditions for producing bioethanol from cocoa biomass, taking into account the complications associated with working with this particular substrate. The objective is to propose viable alternatives that enhance the accessibility and efficiency of the process, thereby promoting the effective use of these agro-industrial residues.

2 Materials and Methods

2.1 Sample collection and processing

2.1.1 Sample collection

The cocoa husks (*Theobroma cacao*) from the CCN-51 clone were collected in April 2020 from a private estate located in the Buena Fe district, Los Ríos Province, Ecuador.

2.1.2 Sample Treatment

Drying and Milling

The cocoa husks were cut into pieces of approximately 1 cm^3 and air-dried for 7 days. Subsequently, they were manually ground using a low-hopper Corona plate mill. The resulting particles were sieved using a USA Standard Test Sieve mesh #18 with 1 mm porosity. Particles smaller than 1 mm were separated and stored in a desiccator.

Removal of Volatile Extractives

This process followed the NREL/TP-510-42619 reference method (Sluiter et al., 2005), in which 10.0000 g of the sieved sample were subjected to a two-stage Soxhlet extraction: first with 200 mL of distilled water for 2 hours, and then with 200 mL of ethanol for an additional 2 hours.

2.2 Biomass Characterization

Characterization was conducted following procedures from AOAC International, ASTM International, NREL, and TAPPI. All analyses were performed in triplicate.

2.2.1 Moisture Content Determination

Moisture content was determined in accordance with AOAC 934.01 (AOAC, 2012). A 1.0000 g sample was weighed into a crucible, placed in a preheated oven at 105 °C, and dried for 3 hours. After drying, the crucible was transferred to a desiccator until it reached room temperature, then weighed. The drying process was repeated in 1-hour intervals until a constant weight was achieved.

2.2.2 Ash Content Determination

Ash content was measured following AOAC 942.05 (Thiex et al., 2012), using incineration at 550 °C. The crucible containing the previously dried sample was placed in a preheated muffle furnace for 5 hours. After cooling in a desiccator to room temperature, it was weighed and recorded. The process was repeated for 1-hour intervals until constant weight was obtained.

2.2.3 Holocellulose Content Determination

A 4.0000 g sample was placed in an Erlenmeyer flask and treated with 300 mL of distilled water, 0.4 mL of glacial acetic acid, and 2.0000 g of sodium chlorite. The flask was heated in a water bath (Memmert) at 75 °C for 1 hour. This process was repeated three times until a whitish coloration was observed. The mixture was then cooled in an ice bath at 10 °C, centrifuged at 3500 rpm for 15 minutes, and vacuum-filtered. The filtered and washed product was dried in an oven at 105 °C for 4 hours, then transferred to a desiccator until room temperature was reached, and weighed. The drying cycle was repeated until a constant weight was reached. Final holocellulose content was determined by the difference in weight between the treated crucible and the empty dried crucible (Nomanbhay et al., 2013).

2.2.4 Cellulose Content Determination

Cellulose content was determined according to ASTM D16-96-95(2019)e1 (ASTM International, 2019). A 2.0000 g holocellulose sample was treated in an Erlenmeyer flask with 10 mL of 17.5 % sodium hydroxide (rested for 5 minutes), followed by an additional 5 mL (rested for 30 minutes), and then 30 mL of distilled water (rested for 1 hour). The mixture was vacuum filtered, washed three times with water-sodium hydroxide solution, followed by 30

mL of water. Then, 5 mL of 10% acetic acid and 50 mL of distilled water were added, and the sample was vacuum filtered again. The residue was dried in an oven at 105 °C for 12 hours, transferred to a desiccator to cool, and weighed until constant mass was reached.

2.2.5 Hemicellulose Content Determination

Hemicellulose content was calculated as the difference between the holocellulose and cellulose content, following Loja Sánchez (2016).

2.2.6 Lignin Content Determination

Lignin content was determined following TAPPI T-222 om-02 (TAPPI, 2002), which assesses acid-insoluble lignin in wood and unbleached pulp. Approximately 1 g of dried cocoa husk sample was placed in a flask with 15 mL of 72% sulfuric acid and stirred at 400 rpm for 1 hour using a ColeParmer mechanical shaker. The sample was transferred to a 250 mL flask with 125 mL distilled water and refluxed for 4 hours. It was then vacuum filtered, washed with 500 mL hot water, dried at 105 °C for 3 hours, cooled in a desiccator, weighed, and re-dried to constant weight.

2.3 Bioethanol Production

2.3.1 Alkaline Pretreatment

Following Jannah and Asip (2015), the milled cocoa husk biomass was treated with 3% sodium hydroxide until reaching a pH of 11. The lignocellulosic biomass was immersed in the solution at a solid/liquid ratio of 1:10 (100 g sample/1000 mL of 3% NaOH), incubated at 121 °C for 90 minutes to enhance biomass swelling and enzymatic accessibility. The sample was filtered and neutralized with distilled water washes and 30% HCl until the filtrate reached pH 5. The biomass was then dried at 60 °C for 24 hours.

2.3.2 Enzymatic Hydrolysis Using Endophytic Fungi *Trichoderma reesei* and *Trichoderma ghanense*

The method, based on NREL TP-510.42629 (Selig et al., 2008), involved cellulase-mediated hydrolysis of cellulose into low-molecular-weight sugars. Enzyme-producing endophytic fungi (*Trichoderma reesei* and T. ghanense) were obtained from the microbial culture collection of the CIBE, Escuela Superior Politécnica del Litoral (ESPOL). Preparation steps included:

- **Re-inoculation:** Three replicates were grown on PDA plates at 26 °C for 7 days until sporulation.
- **Spore Washing:** Spores were removed using 10 mL of saline solution with mechanical agitation.
- **Spore Suspension:** Concentrations were adjusted to 1×10^7 and 1×10^9 spores/mL using a Neubauer chamber.
- **Substrate Preparation and Inoculation:** 50 g of cocoa husk substrate per sample were sterilized at 120 °C for 15 minutes and dried at 60 °C for 24 hours. The moisture content was adjusted to 75% with 35 mL of sterile distilled water. Then, 10 mL of the fungal spore solution (at both concentrations) was added to each flask. Samples were incubated at 25 °C for 10 days.

2.3.3 Alcoholic Fermentation

Saccharomyces cerevisiae yeast was used due to its high fermentative capacity (Van Zyl et al., 2007). Yeast activation involved mixing 0.5 g sugar and 15 g yeast in 75 mL of sterilized distilled water at 28 °C for 20 minutes. The volume was adjusted to 250 mL and added to bioreactors under five scenarios:

- **Scenario 1:** 50 g of alkaline pretreated cocoa husk.
- **Scenarios 2 and 3:** Alkaline pretreated and enzymatically hydrolyzed biomass with T. reesei at 1×10^7 and 1×10^9 spores/mL, respectively.
- **Scenarios 4 and 5:** Same as above but with T. ghanense at 1×10^7 and 1×10^9 spores/mL

Fermentation was anaerobic, with bioreactors sealed and CO_2 released through a purge hose submerged in distilled water. The process lasted 4 days at ambient temperature (∼25 °C) in darkness. After fermentation, the liquid was filtered, allowed to settle in test tubes, and the supernatant was decanted for analysis.

2.4 Ethanol Quantification

Ethanol was quantified using a Thermo Scientific gas chromatograph equipped with a flame ionization detector. The ethanol concentration was expressed in mg% AA and converted to mL of ethanol per gram of cocoa husk biomass. A calibration curve using absolute ethanol was prepared to determine ethanol concentration in the samples (Mansur et al., 2022). Analytical conditions were optimized, as summarized in Table 1.

Table 1. Chromatographic conditions

Equipment	Thermo Fisher gas chromatograph (GC), TRACE GC 1300 Series
Carrier gas	Helium
Flow rate	2 mL/min
Injection volume	1 uL
Injection mode	Split
Injector temperature	180 °C
Column	J&W Scientific DB-FFAP, 60 m × 0.250 mm (ID) × 0.25 μm
Stationary phase	Modified polyethylene glycol acid
Oven temperature	180 °C
Detector	Flame Ionization Detector (FID)
Detector temperature	250 °C

3 Results and Discussion

3.1 Biomass Characterization

The analysis of cocoa husk (*Theobroma cacao*) from the CCN-51 clone was carried out to determine the most relevant parameters for ethanol production, namely: moisture, ash, holocellulose, cellulose, hemicellulose, and lignin. These parameters were used to establish the optimal conditions for pretreatment, enzymatic hydrolysis, and the fermentation process. Their values guided the selection of the most effective procedures to ensure proper ethanolic fermentation.

The moisture content obtained was 11.16%, as shown in Table 2. This value is higher than that reported by Vivanco Carpio et al. (2018), who presented average values of 8.74% for national cocoa and 6.43% for CCN-51 cocoa collected from the province of El Oro. This difference may be attributed to the specific climatic conditions of the mountainous cultivation area in the Buena Fe canton, Los Ríos Province, where the humus-rich soil is suitable for various crops and to the environmental humidity exposure during the transport of the cocoa husk to the laboratory.

The average ash content in the evaluated lignocellulosic biomass was 10.70%, a value comparable to that reported by Villamizar Jaimes et al. (2021) for Colombian cocoa husks of the same variety, with estimated values of 10.77% and 11.39%, depending on whether the sample was untreated or oven-dried. In contrast, Vivanco Carpio et al. (2018) reported an average ash content of 5.54% for Ecuadorian CCN-51 cocoa husks, and 5.14% for national cocoa. Similarly, Castillo et al. (2018) reported 8.59% for this variety of Venezuelan cocoa.

The variation in ash content observed in different studies may be influenced by climatic and edaphic factors. This was demonstrated by Chafla et al. (2016) in a study conducted in various Amazonian provinces on CCN-51 cocoa husks, where it was shown that soil quality, mineral composition, and moisture saturation significantly affect the ash content. Therefore, it can be inferred that the current sample was influenced by the mineral composition of the mountainous soil.

According to the results in Table 2, the average cellulose and hemicellulose contents were 26.08% and 5.38%, respectively, both with a variation coefficient below 2%. Torres (2016) reported similar results, with an average cellulose content of 24.02% for the same matrix, while hemicellulose content averaged 2.23%, though with a high variability (31.44%), indicating inconsistency in results. In that case, the variation in hemicellulose content was attributed to the use of pH adjustment with sulfuric acid, an inorganic compound that may degrade this polysaccharide (Torres, 2016). Likewise, Loayza (2020) confirmed cellulose and hemicellulose contents of 29.09% and 2.97%, respectively, using acid treatment of the biomass.

Table 2. Moisture, ash, holocellulose, cellulose, hemicellulose and lignin content of cocoa shells.

Parameter	$\bar{X}$	σ	C_v
Moisture	11.16%	0.05	0.54%
Ash	10.70%	0.34	2.71%
Holocellulose	31.80%	0.25	0.79%
Cellulose	26.24	0.25	0.09%
Hemicellulose	5.38	0.09	1.66%
Lignin	28.52%	0.75	2.62%

The total lignin content of biomass includes both acid-insoluble lignin (AIL) and, to a lesser extent, acid-soluble lignin (ASL), with the former being more easily identified due to its abundance in lignocellulosic biomass and the use of gravimetric methods. Literature confirms that lignin is the most abundant compound in cocoa biomass, as supported by Encalada and Jácome (2018), who reported an average content of 25.81%, and Vásquez (2010), who found concentrations ranging from 14.6% to 26.38%. Other studies report even higher total lignin contents, exceeding 40%, such as Benalcázar (2018) with 46.61%, and Torres (2016), who reported values ranging from 33.43% to 45.39% based on multiple analyses. This study focused on the determination of acid-insoluble lignin, as it is the predominant polymer in the biomass of interest, while the soluble fraction is typically lost during the extraction of volatile compounds. The average value obtained was 28.52%, as shown in Table 2, with a variation coefficient of 2.62%, confirming the precision of the results.

3.2 Bioethanol Production and Quantification

During alkaline pretreatment of the biomass using 3% sodium hydroxide, physical changes were observed as a result of lignin removal. One noticeable change was biomass swelling and a color transformation from the characteristic brown of dry cocoa husk to black after pretreatment. This was attributed to the breakdown of ester bonds in the plant material and the elimination of reddish lignin. After pH adjustment, the biomass underwent another color change, turning from black to light brown.

Enzymatic hydrolysis was conducted using two different fungi: *Trichoderma reesei* and *Trichoderma ghanense*, with spore concentrations of 1×10^7 and 1×10^9 spores/mL for both species. After 10 days of incubation at room temperature in contact with the biomass inside the bioreactors, greenish coloration was observed on the surface of the biomass, with more prominent growth along the reactor walls-an indication of fungal development. Additionally, biomass swelling was observed, consistent with the conversion of cellulose and hemicellulose into fermentable sugars.

Following enzymatic hydrolysis, alcoholic fermentation was carried out using *Saccharomyces cerevisiae* yeast for 4 days. During this period, intense bubbling was observed at the outlet of the bioreactor, indicating CO_2 emission as a byproduct of alcoholic fermentation. Biomass swelling and abundant yeast growth on the reactor surface were also noted. Upon opening the reactor, a strong odor characteristic of fermentation processes was detected.

Before analyzing the fermented products, a calibration curve was prepared using five ethanol standard concentrations: 0.1, 0.3, 0.5, 0.7, and 0.9% (v/v). The calibration curve yielded a satisfactory correlation coefficient (R = 0.9931). Interpretation of the resulting chromatograms was based on the ethanol elution peak, using retention time and peak area data.

Analysis of the samples showed that **Scenario 1**, which did not include enzymatic hydrolysis, resulted in the highest ethanol yield (0.25% v/v), in contrast to **Scenario 2** (less than 0.1% v/v), **Scenario 3** (below 0.15% v/v), **Scenario 4** (under 0.22% v/v), and **Scenario 5** (below 0.15% v/v), as shown in Table 3.

According to the values obtained in Scenario 1, the results are comparable to those reported by Benalcázar (2018), who achieved an ethanol concentration of 0.57% v/v using alkaline hydrolysis treatment under conditions similar to those in the present study. This confirms that the method is effective for cocoa husk due to its ability to break the bonds linking lignin and hemicellulose chains. However, the yield from biological treatment remains relatively low.

Table 3. Bioethanol production results

Scenario	Sample	Retention Time (min)	Ethanol % v/v
Scenario 1: Alkaline Pretreatment	Blank 1	4.187	0.267
	Blank 2	4.183	0.133
	Blank 3	4.190	0.362
			$\bar{X}$ = 0.254
Scenario 2: Alkaline Pretreatment + *T. reesei* 1×10^7 spores/mL	E2M1	4.185	0.083
	E2M2	4.183	0.145
	E2M3	4.183	0.026
			$\bar{X}$ = 0.084
Scenario 3: Alkaline Pretreatment + *T. reesei* 1×10^9 spores/mL	E3M1	4.185	0.029
	E3M2	4.183	0.045
	E3M3	4.183	0.000
			$\bar{X}$ = 0.024
Scenario 4: Alkaline Pretreatment + *T. ghanense* 1×10^7 spores/mL	E4M1	4.183	0.1256
	E4M2	4.185	0.2251
	E4M3	4.183	0.0559
			$\bar{X}$ = 0.1355
Scenario 5: Alkaline Pretreatment + *T. ghanense* 1×10^9 spores/mL	E5M1	4.185	0.1444
	E5M2	4.185	0.1057
	E5M3	4.187	0.0686
			$\bar{X}$ = 0.1062

The composition of lignocellulosic biomass varies depending on the substrate type. Thus, the levels of cellulose and hemicellulose present determine the efficiency of sugar-to-ethanol conversion. This is supported by the findings of Casabar et al. (2019) in their study on bioethanol production from pineapple peels, which showed that a decrease in reducing sugar corresponds with an increase in ethanol production. This is primarily due to the fermentable sugars derived from cellulose and hemicellulose hydrolysis, which are utilized by *Saccharomyces cerevisiae* during fermentation to produce ethanol.

In this study, the cocoa husk matrix contained 26.24% cellulose and only 5.38% hemicellulose, with the latter potentially limiting the generation of reducing sugars. The availability of hemicellulose for fermentation may have been negatively affected by the applied hydrolysis pretreatment, which disrupts the structural bonds between lignin and carbohydrates, leading to hemicellulose degradation and lignin solubilization. This progressively reduces polymer content for conversion into sugars, as also observed by Sánchez Riaño et al. (2010) in various chemical pretreatment methods for biomass.

Based on these findings, Scenario 1 achieved the highest bioethanol yield under the tested conditions. Nevertheless, the yield was limited by two key factors: the type of pretreatment, as previously described, and the lignin content in the substrate,

as this polymer restricts cellulose and hemicellulose hydrolysis (Ko et al., 2015). Literature suggests that enzymatic hydrolysis using *Trichoderma* spp. could enhance the production of reducing sugars via cellulase enzymes. For instance, López et al. (2014) demonstrated that banana peel biomass yielded up to 5.18% v/v ethanol using *Trichoderma* species, attributed to its 23% cellulose and 23% hemicellulose content.

However, in this study, scenarios treated with *Trichoderma reesei* produced low ethanol yields (0.084% v/v and 0.024% v/v), while *Trichoderma ghanense* yielded 0.1355% v/v and 0.1062% v/v, respectively. These results indicate that biomass composition significantly affects fungal enzymatic performance, particularly due to the limited hemicellulose content. Additionally, the low yields in Scenarios 2 to 5 suggest that other factors, such as the hydrolysis duration and fungal concentration, also influence outcomes. These two variables interact-once cellulose degradation is complete, the fungus may consume the resulting glucose for survival.

Overall, the factors that most significantly impacted ethanol production were biomass composition, pretreatment method, and fungal concentration. In this context, the findings support a negative hypothesis regarding the effectiveness of fungal application on cocoa lignocellulosic residues for ethanol production, due to the low yields obtained with phytopathogenic fungi.

Furthermore, comparing ethanol yields between the scenarios involving *Trichoderma reesei* and *Trichoderma ghanense*, the latter exhibited higher ethanol production.

4 Conclusions

This study provided a well-characterized lignocellulosic matrix with high lignin content and low hemicellulose content, in alignment with previously reported data for the same cocoa husk variety. As such, cocoa husk presents itself as a viable substrate for biomass-to-ethanol conversion processes.

Based on the variables tested during this comparative fermentation study, the optimal conditions for ethanol production were achieved using alkaline pretreatment, with 50 g of biomass incubated at room temperature over a period of 3 days. Scenario 1 yielded the highest ethanol concentration at 0.25% v/v. However, for cocoa husk to be more efficient as a bioethanol feedstock, a higher hemicellulose content is necessary. In combination with cellulose, this would enable the generation of sufficient reducing sugars to significantly enhance ethanol yields through enzymatic hydrolysis with *Trichoderma reesei* and *Trichoderma ghanense*.

Author Contributions

V.P.J.E.: Conceptualization, methodology, resources, supervision, review, and writing-editing. T.Z.Z.: Conceptualization, methodology, project administration, visualization, and original draft writing. L.L.G.R.: Methodology and resources. M.M.M.: Methodology, supervision, validation, review, and writing-editing. J.V.V.: Investigation and resources. V.A.C.M.: Investigation, formal analysis, and validation. F.C.C.C.: Investigation, formal analysis, and validation. D.B.C.: Investigation, formal analysis, and validation. L.V.M.: Investigation, formal analysis, and validation. R.F.E.L.: Investigation and resources.

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