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Evaluation of the Biodegradation Potential of Phytopathogenic Fungi in Sugar Cane (*Saccharum officinarum*) Waste from the Rural Sector of Milagro, Ecuador

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Abstract: In Ecuador, sugarcane (*Saccharum officinarum*) is a grass of great socioeconomic impact due to the employment rate involved in its cultivation and its use as a raw material for obtaining sugar and other derivatives. The industrial processing of the usable sugarcane material generates an excessive amount of waste, including leaves, bagasse, molasses, and other types of organic residues. Waste treatment systems have demonstrated inefficiency in the degradation time with respect to the harmful effects they cause. In this study, the dynamics of two genera of phytopathogenic microorganisms (*Colletotrichum* spp. and *Rhizopus* spp.) in the decomposition of sugarcane organic wastes were tested by analyzing the proximate composition, biodegradation characteristics, microbial incidence, and amino acid content. The results showed that inoculation with a combination of 2.00×10^6 spores/mL of *Colletotrichum* spp. and 2.00×10^6 spores/mL of *Rhizopus* spp., corresponding to treatment T4, led to a higher degree of biodegradation of the residues and aspartic amino acid content, with an incidence of 14.11 mmol/100 g. The amount of amino acids was not closely related to the addition of microorganisms, since the wastes belonging to the control treatment were not recorded as the wastes with the lowest concentration. On the other hand, the different treatments induced variations in the quantification of microorganisms in each biodegraded waste, reporting an average of 5.43×10^4 CFU/g of mesophilic bacteria and 6.52×10^4 CFU/g of fungi with treatment T2. The amounts obtained highlighted the predominance of cycles of increase and decrease in the concentration of microorganisms in a compost according to the stage of compost maturation.

Keywords: sugarcane; *Saccharum officinarum*; *Colletotrichum* spp.; *Rhizopus* spp.; biodegradation; wastes

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1. Introduction

Sugarcane (*Saccharum officinarum*) is a perennial grass native to New Guinea, introduced to the American continent in 1943 through the movement of plants and animals by colonizers. In Ecuador, sugarcane holds great socioeconomic importance as the main raw material for sugar and its derivatives. The sector involves 54 tillage-related and 10 manufacturing companies, generating approximately 6141 jobs nationwide [1]. According to data from the National Institute of Statistics and Census (INEC) [2], in 2022, 116,515 hectares were allocated to sugarcane cultivation, with an average yield of 16.67 tons

per hectare. Guayas province led sugarcane processing with 87% of national throughput, largely due to the activity of Milagro, home to the country's highest-producing sugar mill.

Agroindustrial production generates significant volumes of waste. Improper management of sugarcane residues—such as leaves, bagasse, molasses, and filter cake—remains a global concern, with by-products exceeding 190 million tons annually [3]. Waste treatment systems that promote disposal or recycling help recover nutrients and reduce the environmental impact caused by accumulation or dumping into water sources. However, in Ecuador, a traditionally agricultural country, management practices remain inefficient. The open burning of sugarcane fields after harvest is still common and contributes to biodiversity loss, soil degradation, and air pollution [4]. In this context, current trends in the sugarcane sector are increasingly focused on more sustainable waste management strategies, where the transformation of residues into value-added products, the adoption of circular economy models, and the implementation of cleaner technologies are becoming central. Current trends include the use of sugarcane biomass for biofuel and biogas production, the development of integrated biorefineries, and the cascade utilization of by-products for bioplastics, biofertilizers, and lignin-derived materials [5,6]. Additionally, sustainable field practices such as mulching are being promoted over open burning to improve soil health and reduce emissions [6]. These approaches reflect a growing interest in environmentally sound alternatives for managing sugarcane waste.

In the search for improvements in waste management, new technologies have been tested to reduce degradation time, such as the use of subcritical water and alkaline heat. Subcritical water, in brief, is a chemical method based on thermodynamic principles in which water is heated and pressurized to a point where it remains in a liquid state, although below its critical point [7]. Under these conditions, subcritical water acquires physical and chemical properties similar to those of conventional organic solvents, but without the risks associated with these harmful compounds [8]. On the other hand, chemical methods have the disadvantage of denaturing the biological properties of organic wastes, through the alteration or destruction of molecular structures, since the purpose of these techniques is to reduce the volume of agricultural wastes [9]. Other studies have reported the participation of microorganisms in the decomposition of organic wastes for composting [10].

Heterotrophic microorganisms act as the primary decomposers of organic matter in terrestrial ecosystems, and are considered the drivers of natural ecosystem restoration [11]. Fungi and bacteria are primarily responsible for the physicochemical changes that occur in composting, regulate the rate of conversion of organic wastes, and determine the final quality of compost [12].

Phytopathogenic fungi, classified as plant pathogens due to their ability to colonize plant cells and induce allelochemical precursors of apoptosis, play a key role in plant degradation processes. Although they can cause significant economic losses, studies have shown that the joint participation of fungal consortia formed by *Colletotrichum* sp., *Penicillium* sp., and *Rhizopus* sp. enables the decomposition of plant material and contributes to the nutrient cycle, facilitating recycling, mineralization, and the release of essential compounds into the environment [13]. Given their recognized biodegradation potential, this study focuses exclusively on evaluating the efficiency of *Colletotrichum* sp. and *Rhizopus* sp. spores as biological agents for the decomposition of processed sugarcane residues in the city of Milagro.

The selection of these fungi is based on their natural presence in the local ecosystem and their ability to enhance composting processes by accelerating the degradation of agricultural waste. *Colletotrichum* spp. and *Rhizopus* spp., although known as phytopathogens that cause significant crop losses, also exhibit strong saprophytic capabilities, allowing them to colonize plant by-products. Under nutrient-limited conditions, certain species of

Colletotrichum are capable of increasing the production of reactive oxygen species (ROS), leading to abnormal fungal growth, while some *Rhizopus* species can dehydrate colonized plant tissues and secrete a wide array of extracellular enzymes that facilitate growth on lignocellulosic substrates [14]. Although various microorganisms such as *Trichoderma* spp. and *Aspergillus* spp. have been extensively studied and widely applied due to their recognized degradative potential, there is limited evidence regarding the applicability of *Colletotrichum* and *Rhizopus* species as biodegraders of agricultural residues [13,14]. Therefore, evaluating their potential could provide a foundation for expanding the range of biotechnological applications in organic waste degradation, addressing certain practical limitations observed with other microorganisms, such as the need for pretreated substrates or limited fiber decomposition.

The concept of quality of an agricultural product is closely related to the presence or absence of foreign bodies that can cause contamination, as well as to the study of physicochemical parameters and leachate production that characterize it [15]. The physicochemical composition of a compost produced by the action of exogenous decomposer microorganisms is complex to predict, due to the inherent interaction of the genetic variability of the decomposer microorganisms and environmental factors during waste disintegration. Data mining techniques allow processing large volumes of information in two-dimensional planes and offer a more comprehensive visualization of the dynamics of the synthesizing microorganisms. There are several algorithms and methodologies that focus on the relationship between different attributes, such as the PCA biplot. The application of these algorithms to by-products made from organic waste promises to positively revolutionize waste management [16].

The main objective of this article was to use the multivariate PCA biplot technique to determine the dynamics of two types of phytopathogenic microorganisms in the decomposition of organic sugarcane waste through the analysis of the proximate composition of the compost obtained. In addition, the biodegradation characteristics, microbial incidence, and amino acid content were evaluated using the Duncan test ($p < 0.05$) to assess the efficiency and effectiveness of the decomposers in the biodegradation process compared to the control treatment, where no new microorganisms were inoculated. The Duncan test was chosen for its ability to detect differences between treatments with greater sensitivity for balanced samples, making it suitable for biological studies with hierarchical responses. Unlike Tukey or Bonferroni, which are more conservative, Duncan allows a more flexible comparison of treatment effects without being excessively restrictive [17].

2. Materials and Methods

2.1. Biological Material

In this study, *Mangifera indica* (mango) and *Musa acuminata* (banana) fruits with symptoms of post-harvest soft rot and anthracnose, respectively, were used exclusively as a source for isolating *Colletotrichum* spp. and *Rhizopus* spp. These fruits were selected due to the high prevalence of these fungi on their surface, facilitating a more efficient isolation compared to sugarcane residues, where their natural presence is less significant [13,18]. However, their use was limited to obtaining the inoculum and did not play a role in the experimental production process. All samples were purchased at the Wholesale Market "Montebello," Guayaquil, Ecuador, and were subsequently washed with distilled water and disinfected with a 2% sodium hypochlorite solution for 3 min.

2.2. Isolation of Phytopathogenic Fungi

Colletotrichum spp. and *Rhizopus* spp. strains were isolated and purified in Petri dishes with PDA (Papa Dextrose Agar) culture medium from visible anthracnose and soft rot

lesions by *Colletotrichum* spp. and *Rhizopus* spp. on previously disinfected mangoes and bananas. This process was carried out at the Research and Development Laboratory of the Ecuahidrolizados Industry, located in Guayaquil, Ecuador. Monosporic cultures were obtained following the methodology described by Than et al. [19].

The plates were incubated at 25–28 °C for 5 days under dark conditions to promote fungal growth. Colonies were selected based on macroscopic characteristics (color, texture, growth rate) and confirmed by microscopic observation of conidia morphology. *Colletotrichum* spp. were identified by the presence of fusiform conidia, while *Rhizopus* spp. were distinguished by the development of sporangia containing sporangiospores and extensive mycelial growth. To ensure purity, monosporic cultures were subcultured on fresh PDA plates before use.

2.3. Propagation Solution

A 1 L solution high in sugars was prepared as a carbon and energy source to maximize the fungal synthesis of secondary metabolites. The formulation of the solution consisted of distilled water enriched with 40% glucose and guar gum. Subsequently, 300 mL of propagation solution was poured into 500 mL Erlemeyer flasks and the vessels were autoclaved (Yamato Model SM311, Santa Clara, CA, USA) at 121 °C for 15 min. After sterilization, the flasks were incubated at 28 °C (BIOBASE Biochemical Incubator Model BJPX-B, Jinan, China) for 24 h to check sterility [20].

The Petri dishes with the monosporic cultures were washed using 100 mL of distilled water, with the intention of capturing the spores of the phytopathogenic fungi. The spore suspensions resulting from the washing were transferred to Erlenmeyer flasks, according to each type of fungus, and distilled water was added until reaching a total volume of 1 L with a concentration of 4.00×10^6 esporas/mL.

2.4. Production of Biodegraders Based on Fungal Spores of Colletotrichum spp. and Rhizopus spp.

Following the method described by Valenzuela et al. [21], biodegraders were prepared based on the propagation solutions. The biodegraders were obtained according to the following formulation:

Biodegrader 1 (B1): 1 L of propagation solution with a concentration of 4.00×10^6 esporas/mL (*Colletotrichum* spp.) + 1 L of sterile sugarcane juice.

Biodegrader 2 (B2): 1 L of propagation solution with a concentration of 4.00×10^6 esporas/mL (*Rhizopus* spp.) + 1 L of sterile sugarcane juice.

Biodegrader 3 (B3): 1 L of propagation solution with a concentration of 4.00×10^6 esporas/mL (2.00×10^6 esporas/mL *Colletotrichum* spp. + 4.00×10^6 esporas/mL *Rhizopus* spp.) + 1 L of sterile sugarcane juice.

The biopreparations were homogenized by shaking and the mixture was allowed to stand for 24 h.

The selection of a 4.00×10^6 spores/mL concentration for B1 and B2 was based on its effectiveness in fungal degradation studies, as concentrations within the 10^6 range have been widely used in experimental assays. Previous research has demonstrated that spore concentrations between 1×10^6 and 5×10^6 spores/mL enhance fungal activity and biodegradation efficiency, balancing practicality and biological effectiveness [13,18,22].

In Biodegrader 3 (B3), the combination of 2.00×10^6 spores/mL of *Colletotrichum* spp. and 4.00×10^6 spores/mL of *Rhizopus* spp. resulted in a final concentration of 4.00×10^6 spores/mL due to concentration adjustments during mixing. Since concentration is a ratio, it does not sum linearly; instead, it is influenced by the total number of spores and the final volume. The selected values ensured consistency with B1 and B2 while maintaining optimal fungal activity for biodegradation.

2.5. Field Experimentation

The experiment was carried out at the Sofia farm, located in the city of Milagro, Ecuador. Four treatments were implemented, each treated in triplicate, assigned as follows: T1 (control treatment), T2 (treatment using B1), T3 (treatment using B2), and T4 (treatment using B3). The sugarcane residues used were collected from a local farmland in the city of Milagro. The residue mixture consisted of the following proportion: 25% leaves, 60% bagasse, and 15% cachaza.

To initiate the process, 2.5 kg of sugarcane waste was weighed for each test and placed in 5 kg capacity plastic containers. These containers were designed with holes in the lid and base to allow aeration, and had a plate at the base for leachate collection. The waste was inoculated with the corresponding treatment and incubated at a temperature of 25 °C and relative humidity between 95 and 100% for 30 days.

2.6. Biodegradation Tests

At the end of the incubation stage, the amount of leachate obtained was estimated by measuring the volume of liquids accumulated in the dish of each experimental vessel. In addition, the pH of the leachate was measured using a calibrated pH meter (Model PCE-PH22, Meschede, Germany).

In addition, the biodegradation of agricultural residues induced by each treatment was evaluated. For this purpose, the sugarcane residues were weighed without leachate and the quantitative method of mass loss was applied, using a digital scale (Torrey PCR-40, Mexico) [23]. Equation (1). Calculate biodegradation by quantitative mass loss method.

$$\text{Biodegradation} = \frac{W_o - W_f}{W_o} \times 100 \quad (1)$$

where

W_o = initial mass of waste (g);

W_f = final mass of waste without leachate (g).

2.7. Laboratory Processing

Samples of 50 g of degraded sugarcane residues were taken from each container for processing at the Research and Development Laboratory of the Ecuahidrolizados S.A.S. Company.

2.7.1. Determination of Proximate Composition

The analysis of the proximate composition of the degraded residues was carried out by calculating the moisture, ash, organic carbon, organic matter, C/N ratio, and total nitrogen content. All standard methodologies were procedures adapted from the Association of Official Analytical Chemists [AOACs] [24].

2.7.2. Determination of Amino Acids

The amino acid (AA) content of the sugarcane residues with the highest degree of degradation was evaluated. The amino acids evaluated were glutamic acid, aspartic acid, glycine, proline, cysteine, tyrosine, valine, methionine, lysine, isoleucine, leucine, and phenylalanine. The determination was performed by high performance liquid chromatography (HPLC), following the methodology of Ya-Qin et al. [25].

2.7.3. Determination of Microbiological Parameters

The microbiological parameters of the biodegraded sugarcane residues, both with and without biodegrader, were analyzed to determine the population dynamics of the differ-

ent microorganisms. The analyses included the quantification of mesophilic aerobic heterotrophic bacteria and fungi, following the procedures established by the AOAC [26–28].

2.8. Statistical Analysis

2.8.1. PCA Biplot

The proximate composition results were subjected to data mining techniques, such as the PCA biplot, using R software ver. 4.4.0. software (R Core Team, Vienna, Austria).

Principal component analysis (PCA) is one of the most widely used statistical techniques in exploratory analysis and data mining. This method focuses on identifying the orthogonal principal components that explain most of the variability in a data matrix. The new rotated variables, called principal components, retain most of the information from the original set in a matrix of lower dimensionality and can be interpreted quantitatively as possible sources of variation, thus facilitating the analysis of the interrelationships that exist in complex data sets [29–32].

The $n \times p$ -dimensional data matrix X consists of p n-dimensional vectors $\mathbf{x}_1, \ldots, \mathbf{x}_p$. The linear combinations of these vectors are given by the mathematical expression $\sum_{j=1}^{p} a_j x_j = Xa$, where a is a vector of constants $\boldsymbol{a}_1, \boldsymbol{a}_2, \ldots, \boldsymbol{a}_p$. To maximize the variance, it is necessary to impose the constraint that the vectors have a unit norm. Derivation with respect to the vector and subsequent equating with the null vector generates the following formula [33]. Equation (2). The eigenvalues of the covariance matrix are as follows:

$$\mathrm{Sa} - \lambda \mathrm{a} = 0 \Leftrightarrow \mathrm{Sa} = \lambda \mathrm{a} \tag{2}$$

where

S = covariance matrix;

λ = corresponding eigenvalue;

a = eigenvector (unit norm).

The covariance matrix is decomposed in terms of its singular values and vectors. The singular value decomposition (SVD) of the column-centered data matrix X is given by [34]. Equation (3). The DVS of the covariance matrix is as follows:

$$(n-1)\mathrm{S} = X^{*\prime X^*} = (\mathrm{ULA}')'(ULA') = ALU'ULA' = AL^2A' \tag{3}$$

where

L^2 = diagonal matrix with the singular values squared;

L = singular values of Y;

U y A = matrices with orthonormal columns.

For a PCA to be useful for dimensionality reduction, the dominant contribution of a small number of principal components (PCs) to the total variance must be satisfied. The usefulness of the PCA for a target data set is given by [31,32,35], as follows:

$$x_\alpha = \frac{\lambda_\alpha}{\sum_{\alpha=1}^{f} \lambda_\alpha}$$

The visual representation of the PCA biplot, mathematically expressed as $X_{[I,P]} = \{x_{ij} | \mathrm{i} = 1, \ldots, 1; \mathrm{j} = 1, \ldots, \ldots, \mathrm{P}\}$, presents the individuals in rows and the variables in columns. In this expression, each $\boldsymbol{x}_{ij}$ comprises an element of the matrix X in row i and column j [33,34].

2.8.2. Descriptive Statistics

The results related to biodegradation characteristics, microbiological parameters, and amino acid content were studied by analysis of variance (ANOVA). Comparison of means

was performed with Duncan's test ($p < 0.05$). The analysis was carried out in SPSS software ver. 26.0.0.0 (IBM Corp., New York, NY, USA).

3. Results and Discussion

The purpose of this study was to analyze, by means of statistical methods, the efficiency of the use of phytopathogenic fungi as accelerators of sugarcane residue biodegradation. The analysis of the dynamics exerted by natural decomposers was carried out in triplicate. The coding used to distinguish the biodegraded residue samples had the following pattern:

P1–P3: Waste samples to which T1 treatment was provided.
P4–P6: Waste samples to which T2 treatment was provided.
P7–P9: Waste samples to which T3 treatment was provided.
P10–P12: Waste samples to which T4 treatment was provided.

3.1. Biodegradation Characteristics

The biodegradation characteristics of the sugarcane residues based on the treatments applied are presented in Table 1. The residues treated with treatment T4 presented the highest biodegradation rate (55.81%) and the highest leachate production (95.70 mL), followed by treatment T3, whose biodegradation and leachate production rates were 47.76% and 61.30 mL, respectively. On the contrary, the residues corresponding to the control treatment, in which no phytopathogenic fungi were added, presented the lowest decomposition rates (30.90%) and leachate production (33.90 mL).

Table 1. Biodegradation characteristics of sugarcane residues treated with phytopathogenic fungi.

Characteristic	Treatments			
	T1	T2	T3	T4
Biodegradation (%)	$30.90^{a} \pm 0.42$	$38.19^{b} \pm 0.23$	$47.76^{c} \pm 0.36$	$55.81^{d} \pm 0.56$
Leachate production (mL)	$33.90^{a} \pm 0.28$	$56.30^{b} \pm 0.55$	$61.30^{c} \pm 0.36$	$95.70^{d} \pm 0.26$
pH of leachate	$3.10^{a} \pm 0.28$	$3.50^{c} \pm 0.55$	$3.45^{b} \pm 0.21$	$4.30^{d} \pm 0.13$

Note. Values indicate the yield mean ± SE (standard error); Different letters within a column indicate significant differences according to the Duncan test ($p < 0.05$). T1: Control treatment; T2: Treatment based on a concentration of 4.00×10^6 spores/mL of *Colletotrichum* spp.; T3: Treatment based on a concentration of 4.00×10^6 spores/mL of *Rhizopus* spp. concentration; T4: Treatment based on a concentration of 2.00×10^6 spores/mL of *Colletotrichum* spp. + on a concentration of a 2.00×10^6 spores/mL of *Rhizopus* spp.

Several studies have associated the production of leachates and the pH of these soluble compounds with the stage of decomposition and the release of secondary metabolites. Similar research was conducted by Valenzuela et al. [20] in 2019, in which the best percentage of decomposition of banana rachis was obtained with the application of a solution based on the combination of 1.75×10^6 spores/mL of *Colletotrichum gloeosporioides* and 1.75×10^6 spores/mL of *Rhizopus stolonifer*. In 2020, Valenzuela et al. [21] used *C. gloeosporioides* and *R. stolonifer* to improve the biodegradation of cocoa shells and increase the nutritional content of the edible fungus *Pleurotus ostreatus*. In this case, the treatment formulated with a concentration of 2.50×10^6 spores/mL of *C. gloeosporioides* showed the highest rates of biodegradation and leachate production, with values of 57.18% and 84 mL, respectively.

The metabolic capabilities of *Colletotrichum* spp. and *Rhizopus* spp. responsible for the breakdown of cell structures and plant necrosis in various crops have been reported previously. Metabolite production is influenced by environmental factors such as temperature, pH, and nutrient availability. Reveglia et al. [36] reported that the diversity of *Colletotrichum* spp. lifestyles varies from necrotrophic to hemibiotrophic, with 189 secondary metabolites

characterized so far. Sivaramakrishnan et al. [37] used a *Rhizopus* spp. strain for the production of nutrient-free anaerobic fermentative biohydrogen from rice bran. Rice bran residues treated with *Rhizopus* spp. removed up to 38% of the lignin from the feedstock, increasing the efficiency of the process by 1.7%.

Numerous factors influence the degree of degradation and decomposition time of an agricultural residue; among the most determining factors are the nature and chemical composition of the residue, as well as the conditions initially provided for microbial activity. Bohorquez et al. [38] reported that agro-industrial sugarcane by-products subjected to decomposition for 42 days did not acquire optimal conditions for handling and application in the field. In Ethiopia, Kassa et al. [39] reported that composting coffee by-products for up to 70 days was essential to obtain high quality compost.

3.2. Proximate Composition Statistical Algorithm

Figure 1 shows the biplot principal component analysis (PCA) that allowed grouping the samples of degraded sugarcane residues, with and without the inoculation of phytopathogenic fungi, and relating them to their physicochemical characteristics. Plan 1–2 shows the formation of three clusters according to six vectors: moisture, ash, organic nitrogen, organic matter, organic carbon, and the carbon-to-nitrogen ratio. The size of each cluster was determined by the number of residue samples that exhibited the highest correlation with each other. The interpretation indicates that the samples that make up each group are closely related to each other in terms of the quantitative variables measured. In contrast, the groups that are farther apart imply significant differences in their physicochemical composition.

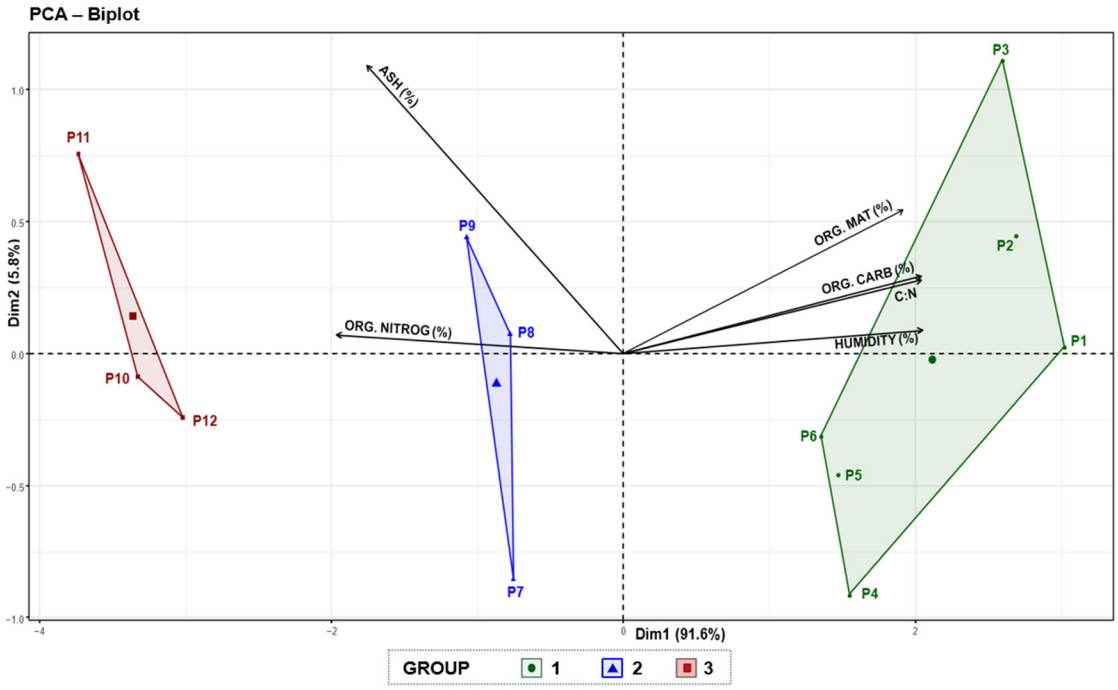

Figure 1. Two-dimensional projection of residue samples on principal components 1 and 2. Note. Each dot represents a waste sample coded by its respective treatment; the vectors represent the proximate composition indicators: Org. nitrogen (%), organic nitrogen (%); Org. Mat, organic matter (%); Org. carb (%), organic carbon (%); C:N, carbon to nitrogen ratio.

In the factorial graph, the accumulated inertia of the two-dimensional representation amounted to 97.4%, with 91.6% of the data variability distributed in the first dimension and 5.8% in the second dimension. The conformation of the clusters was as follows: Cluster

1 (green color), constituted by six samples of degraded residues (P1, P2, P3, P4, P5, P6), corresponding to degraded residues without inoculation and residues inoculated with *Colletotrichum* spp. belonging to treatments T1 and T2, respectively; Cluster 2 (blue color), formed by three samples of processed residues (P7, P8, P9), corresponding to residues inoculated with the phytopathogenic fungus *Rhizopus* spp. from treatment T3; and Cluster 3 (red), composed of three samples of degraded residues (P10, P11, P12) inoculated with the combination of *Colletotrichum* spp. and *Rhizopus* spp. from treatment T4.

The results from the principal component analysis (PCA) reveal distinct patterns in the proximate composition of the degraded residues based on the phytopathogenic fungi used. Cluster 1, which includes residues without inoculation and those treated with *Colletotrichum* spp., exhibits higher levels of organic matter, organic carbon, the carbon–nitrogen ratio, and moisture, indicating incomplete decomposition. Cluster 2, consisting of residues inoculated with *Rhizopus* spp., shows a stronger correlation with organic nitrogen, suggesting a different degradation pathway or nitrogen assimilation process. Cluster 3, where residues were treated with a combination of *Colletotrichum* spp. and *Rhizopus* spp., is associated with higher ash content and organic nitrogen levels, reflecting advanced degradation and mineralization stages. These relationships highlight the differential effects of fungal treatments on the decomposition dynamics of sugarcane residues.

Additionally, Cluster 1 was determined to have the lowest biodegradation index because it is more closely associated with higher values of organic matter and organic carbon. This indicates that the samples in this cluster retain a greater proportion of biodegradable material, suggesting lower degradation efficiency. In contrast, Clusters 2 and 3 show higher associations with organic nitrogen and ash content, which are indicative of more advanced biodegradation stages.

In one study, Wichuk and Mccartney [40] emphasize the importance of evaluating the stability and maturity of a compost, since an immature and unstable compost has the potential to cause a series of problems in crops, such as self-heating, odor emission, inhibition of germination, and phytotoxicity. Within the classification of compost maturity tests, classical chemical tests include evaluations of the carbon/nitrogen ratio, organic matter, humification parameters, exchange capacity, pH, and spectroscopy, among others [41].

According to established criteria for estimating the quality of a compost, the maturity and stability of a compost are delimited by certain conditions, such as moisture 30–60%, the C/N ratio in the range 10:1 to 20:1, organic matter 30–60% on a dry basis, total nitrogen between 1.0 and 2.5%, and phosphorus 0.40–1.20, among others. The amount of ash is an indicator of mineral composition that also provides information about the efficiency of the composting process; an adequate level of ash can indicate that degradation has been completed and the composting process has been efficient. In addition, several studies mention that a low C/N ratio is related to a stabilized product [42]. Based on these criteria, treatments T1 and T2, represented in the residues grouped in Cluster 1, showed the lowest biodegradation index, because they showed low levels of ash and high concentrations of biodegradable organic matter, moisture, organic carbon, and a C/N ratio, indicating that the raw material is still in an active state of decomposition. In contrast, treatment T4, in which *Colletotrichum* spp. and *Rhizopus* spp. were combined, exhibited the best levels of biodegradation in the sugarcane residues, with lower rates of the carbon–nitrogen ratio, moisture, and organic carbon, and the highest levels of ash and organic nitrogen.

In the research conducted by Bohórquez et al. [38], the experimental design for obtaining compost from sugarcane agro-industrial by-products included the formulation of different proportions of cachaza, bagasse, and vinasse. The authors observed a negative trend of most of the nutrients at high moisture levels, which highlighted the relevance of moisture control on the quality of a compost.

In a previous study in Bangladesh, Sarwar et al. [43] indicated that the physicochemical properties and nutritional composition of a compost are substantially influenced by the type of organic waste and the composting techniques employed. In addition, they indicated that a high fraction of biodegradable materials at the end of the process is an index of microbial activity and, therefore, instability. In Ethiopia [39], a compost based on coffee by-products treated for 72 days was characterized physico-chemically by a lower than ambient temperature, optimum humidity, high cation exchange capacity, high available phosphorus content, low organic matter, and low C/N ratio.

3.3. Amino Acid Composition in Biodegraded Residues

Table 2 presents the amino acid composition of sugarcane residues subjected to biodegradation. In particular, aspartic acid from residues treated with the combination of *Colletotrichum* spp. and *Rhizopus* spp. spores was the amino acid with the highest incidence, with a mean of 14.11 mmol/100 g. On the other hand, in general, cysteine was the amino acid that occurred in the lowest proportion in all the biodegraded residues.

Table 2. Amino acid composition of biodegraded sugar cane residues.

AA	Concentration (mmol/100 g)			
	T1	**T2**	**T3**	**T4**
GLU	$10.71^{d} \pm 0.12$	$10.39^{c} \pm 0.02$	$9.04^{b} \pm 0.03$	$8.10^{a} \pm 0.07$
ASP	$4.18^{b} \pm 0.02$	$2.83^{a} \pm 0.05$	$4.89^{c} \pm 0.03$	$14.11^{d} \pm 0.12$
GLY	$9.62^{a} \pm 0.07$	$9.67^{a} \pm 0.14$	$11.27^{b} \pm 0.03$	$11.73^{c} \pm 0.14$
PRO	$6.42^{b} \pm 0.07$	$6.37^{ab} \pm 0.14$	$6.23^{ab} \pm 0.45$	$5.94^{a} \pm 0.07$
CIS	$0.26^{a} \pm 0.06$	$0.54^{b} \pm 0.15$	$0.57^{b} \pm 0.06$	$0.27^{a} \pm 0.05$
TIR	$2.57^{a} \pm 0.14$	$2.64^{a} \pm 0.18$	$2.6^{a} \pm 0.02$	$4.67^{b} \pm 0.09$
VAL	$8.51^{d} \pm 0.10$	$8.03^{c} \pm 0.13$	$7.84^{b} \pm 0.06$	$7.55^{a} \pm 0.06$
MET	$0.87^{c} \pm 0.09$	$0.84^{c} \pm 0.03$	$0.61^{b} \pm 0.06$	$0.27^{a} \pm 0.03$
LIS	$7.82^{a} \pm 0.25$	$8.19^{b} \pm 0.14$	$9.01^{c} \pm 0.12$	$8.29^{b} \pm 0.12$
ISO	$4.78^{b} \pm 0.04$	$4.71^{b} \pm 0.23$	$4.87^{c} \pm 0.05$	$4.07^{a} \pm 0.45$
LEU	$7.01^{a} \pm 0.12$	$7.28^{b} \pm 0.14$	$7.34^{b} \pm 0.09$	$6.87^{a} \pm 0.14$
PHE	$0.99^{a} \pm 0.05$	$1.07^{a} \pm 0.10$	$1.37^{c} \pm 0.07$	$1.21^{b} \pm 0.17$

Note. AA: Amino acids; GLU: Glutamic acid; ASP: Aspartic acid; GLY: Glycine; PRO: Proline; CIS: Cysteine; TIR: Tyrosine; VAL: Valine; MET: Methionine; LIS: Lysine; ISO: Isoleucine; LEU: Leucine; PHE: Phenylalanine. Different letters within the same row indicate significant differences between treatments according to Duncan's test ($p < 0.05$).

On the other hand, the amount of amino acids was not directly related to the addition of microorganisms, since the control group of residues, which did not receive the application of phytopathogenic fungi, reported a higher content of amino acids than the T2 treatment, in which *Colletotrichum* spp. spores were added. In addition, the amino acid indices showed significant differences in the concentrations between each treatment.

The amino acid content in compost, in addition to indicating the degree of organic matter decomposition, can directly influence its agronomic quality. Amino acids such as aspartic acid, glutamic acid, and glycine act as precursors of organically bound nitrogen readily assimilable by soil microbiota and may also stimulate microbial activity and plant development. Several studies have shown that the amino acids present in compost or organic biofertilizers can improve nutrient uptake efficiency, promote chlorophyll synthesis, and enhance plant resistance to abiotic stress [44]. Therefore, their presence in the final

compost product could contribute to improved soil fertility, enhanced soil structure, and more sustainable agricultural practices.

Fermentation time is a key factor in the decomposition and stabilization of nitrogenous compounds, affecting the final amount of amino acids in a biodegraded product. However, in this experiment, all treatments were fermented for the same period due to equipment availability constraints and to maintain a controlled variable framework. This was a study limitation, as varying multiple experimental factors would have increased complexity and hindered the primary objective of evaluating the effect of fungal inoculation on sugarcane residue degradation.

The disparities in the influxes suggest that additional factors influenced the trends, including the specific conditions of the biodegradation process, biological interactions between microorganisms, microbial activity, and the initial composition of the organic waste, among others.

Amino acids are key determinants of compost quality. These functional molecules can stimulate positive effects on plant growth characteristics, including nutrient uptake, PSII (Photosystem II) quantum efficiency, translocation, and biomass production [45]. Recent studies have shown that amino acids are more critical molecules for crops than inorganic nitrogen [46].

Baca et al. [47] examined the amino acid content of two types of compost made from cotton waste as a raw material. Samples of the substrates were evaluated at the end of the thermophilic, mesophilic, and curing stages. The results showed an increase in the total amount of amino acids with time. Regarding aspartic and glutamic acids, their concentration increased at each stage in compost 1 and decreased in compost 2. The changes in amino acid composition were associated with variations in the composition of the microbial population.

Yao et al. [48] characterized the amino acid profile during the co-composting of corn straw and cattle manure. The results coincided in that the total amino acid content decreased during the thermophilic stage; however, at the end of the composting process, a 1.5-fold increase in the total amino acid content was observed, highlighting the relevance of evaluating the appropriate fermentation times.

3.4. Microbiological Parameters

The population dynamics of microorganisms, specifically aerobic mesophilic bacteria and fungi, present in the biodegraded waste are detailed in Table 3. The table shows the mean concentrations of the microorganisms in each type of waste according to the treatment applied. In general, the highest microbial population density was recorded in the waste treated with treatment T1 (control), while the lowest microbial population density was reported in the waste biodegraded with treatment T2.

Table 3. Quantification of microorganisms found in biodegraded wastes.

M.O.	Quantity (UFC/g)			
	T1	**T2**	**T3**	**T4**
Aerobic mesophilic bacteria	$(5.38 \times 10^6)^{c} \pm 0.07$	$(5.43 \times 10^4)^{a} \pm 0.45$	$(1.40 \times 10^5)^{a} \pm 0.45$	$(5.30 \times 10^5)^{b} \pm 0.40$
Fungi	$(2.50 \times 10^6)^{c} \pm 0.50$	$(6.52 \times 10^4)^{a} \pm 1.17$	$(2.73 \times 10^6)^{a} \pm 0.46$	$(3.03 \times 10^5)^{a} \pm 0.45$

Different letters within the same row indicate significant differences between treatments.

The sugarcane residues with the highest degree of maturation, corresponding to treatment T4, reported an average of 5.30×10^5 CFU/g of aerobic mesophilic bacteria

and a rate of 3.03×10^5 CFU/g of fungi. The combined effects of interacting microbial communities have given improvements in waste biodegradation [49]. The dynamics of bacterial and fungal concentrations in a decomposition process follow a characteristic cycle of increasing and decreasing according to each stage. This cycle directly affects the maturation and final stability of the by-products [49,50]. In this context, the amount of microorganisms in the most degraded waste may also be indicative of a more advanced stage compared to other wastes.

Although microbial counts and amino acid profiles were analyzed separately due to their differing nature and scale, their interpretation complements the results obtained from the principal component analysis (PCA). In the biplot (see Figure 1), treatments positioned closer to vectors such as ash and nitrogen (e.g., samples P10 to P12 corresponding to T4) indicate a higher degree of organic matter transformation and greater compost maturity. This clustering can be indirectly associated with increased microbial activity, as effective decomposition processes are typically driven by microbial metabolism.

The synergistic effect resulting from the coexistence of bacteria and fungi in a biological compound is known to be the factor that increases degradation kinetics. However, the intensive interaction of microorganisms induces a relatively high degree of metabolic activity, which in turn can trigger undesirable natural self-heating processes. The main damages resulting from self-heating include large volumes of material due to the rupture of compost containers, odor production, attraction of disease vectors, capture of flammable gases, and fires, among others. Microbial balance is essential to obtain a stable humus, avoiding the dominance of anaerobic bacteria, whose nature is associated with contamination and bad odors [51].

The results of this study suggest a biodegradation mechanism in which *Colletotrichum* spp. and *Rhizopus* spp. act complementarily in the decomposition of sugarcane residues. Colletotrichum spp. facilitates the initial degradation through lignocellulolytic enzymes, weakening the biomass structure, while *Rhizopus* spp. accelerates the conversion of soluble compounds into simpler metabolites, promoting mineralization. The PCA analysis revealed that the combination of both fungi (T4) resulted in the highest degradation, reflected in an increase in ash content and organic nitrogen, indicators of greater conversion of organic matter into stable compounds. In contrast, individual treatments and the control retained higher organic matter content, indicating a lower degree of decomposition.

There are several methods to optimize the biodegradation of agricultural residues, such as the inoculation of beneficial microorganisms, the application of biopreparations, and the biological pretreatment with microorganisms or ligninolytic enzymes, which accelerate the decomposition of lignocellulosic biomass and improve compost quality through humus formation and nutrient release, although some of these methods require specific conditions [10,23,38]. On the other hand, physicochemical methods such as pyrolysis, gasification, or ultrasonic technology enable the transformation of residues into energy-valued products, although they involve higher energy consumption and the need for specialized equipment [52]. In this context, the combined application of phytopathogenic fungi proposed in this study constitutes an eco-efficient strategy that accelerates biodegradation under controlled conditions and aligns with sustainable agro-industrial waste management practices.

4. Limitations and Future Perspectives

Previous studies discussed in this research provide a foundation for highlighting key advantages and limitations. The strategic selection of phytopathogenic fungi from tropical fruits, followed by a controlled combination of treatments under uniform concentration and bioreaction conditions, facilitated a differential analysis of sugarcane residue

degradation efficiency. Additionally, the use of PCA for dimensionality reduction allowed for the identification of underlying degradation patterns that would not be easily captured through simple post hoc tests. This approach not only ensured the reliability of experimental replicates but also identified the variables contributing the most to variance explained by the principal components. However, some limitations of this study include the absence of molecular identification techniques for strain characterization and the lack of evaluation of additional environmental factors that could influence fungal activity. Future research should focus on optimizing inoculum concentrations and assessing long-term biodegradation dynamics under variable conditions.

The biodegradation (fermentation) process applied in this study is based on the growth of *Colletotrichum* spp. and *Rhizopus* spp. on an aerated solid matrix, with controlled humidity and in the absence of a free liquid phase, which characterizes the system as a solid-state fermentation (SSF). The main parameters that influence the biodegradation of lignocellulosic residues under these conditions include the following: time, temperature, pH, moisture, oxygen availability, the carbon-to-nitrogen ratio, the physical structure of the substrate, and the inoculation rate [13,14,20,49]. In this study, humidity and incubation time were primarily controlled in order to preliminarily assess the biodegradation efficiency within a stable system. Other parameters were not varied in order to maintain experimental consistency and reduce sources of variability. However, future studies could include analysis of substrate particle size effects as well as supplementation with carbon- and nitrogen-enriched sources. Such investigations would help optimize process conditions and contribute to the development of sustainable and innovative strategies for agro-industrial waste biodegradation.

5. Conclusions

The physicochemical characteristics of each compost exhibited significant differences depending on the treatment applied, allowing for an assessment of their maturation stages. The combination of *Colletotrichum* spp. and *Rhizopus* spp. (T4) resulted in the highest biodegradation efficiency, as evidenced by greater mineralization and lower organic matter content. PCA analysis confirmed distinct degradation patterns, where T1 and T2 retained higher organic matter levels, indicating incomplete decomposition, while T3 and T4 showed advanced biodegradation stages, reflected in higher ash and organic nitrogen content. Additionally, no consistent trend was observed in amino acid variations across treatments, as different classes exhibited distinct behaviors. The microbial dynamics in biodegraded residues revealed that microorganism abundance varies significantly with compost maturity and species-specific biological factors. The novelty of this study lies in the selection of fungal strains from tropical fruit residues, demonstrating their adaptability to sugarcane waste biodegradation. These findings highlight the role of phytopathogenic fungi as effective biodegraders, offering a sustainable alternative for organic waste management. Future research should focus on optimizing inoculum concentrations and environmental conditions to further enhance biodegradation efficiency.

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