Dual Fungal Inoculation Accelerates Degradation Kinetics and Enhances Compost Maturity in Sterilized Sugarcane Bagasse

Nabaroon Das[1]; Dr. Adlene Sangeeth[2]

[1]JSS Private School, Dubai, UAE
[2]JSS Private School, Dubai, UAE

Publication Date: 2026/06/26

Abstract: Sugarcane bagasse is one of the most abundantly produced lignocellulosic agricultural residues globally, yet its high lignin content and characteristically elevated carbon-to-nitrogen (C:N) ratio—often exceeding 60:1—present substantial barriers to efficient microbial decomposition and timely compost stabilization. While previous research has largely pursued environmental optimization or single-enzyme interventions as strategies to accelerate composting, the potential of deliberately engineered fungal consortia to improve both degradation kinetics and compost quality remains poorly characterized. We hypothesized that the simultaneous introduction of *Phanerochaete chrysosporium* and *Trichoderma viride*—two fungi with complementary but non-overlapping substrate specificities—would generate synergistic improvements in both the rate of organic carbon mineralization and final compost maturity relative to either fungus inoculated alone. To test this, sterilized sugarcane bagasse was inoculated under controlled static conditions at 30°C, with measurements of organic carbon, lignin, total nitrogen, C:N ratio, phenolic content, and the germination index recorded at Day 0, Day 30, and Day 60. Organic carbon loss followed first-order kinetics, and the dual-inoculation treatment yielded the highest degradation rate constant ($k = 0.0090\ \text{day}^{-1}$), representing a 6.9-fold improvement over the uninoculated control ($k = 0.0013\ \text{day}^{-1}$) and a Synergy Index of 1.11, confirming genuinely cooperative rather than additive enhancement. By Day 60, the mixed treatment had reduced the C:N ratio from 70:1 to 17:1, decreased phenolic content from 21 mg/g to 0.4 mg/g, and achieved a germination index of 88%—surpassing the widely accepted 80% threshold for agricultural application. One-way ANOVA confirmed highly significant treatment effects ($F^{3,8} = 214.76$, $p < 0.0001$, $\eta^2 = 0.987$). These findings demonstrate that targeted fungal co-inoculation represents a scientifically grounded and scalable approach for managing lignocellulosic agricultural waste.

Keywords: *Sugarcane Bagasse; Lignocellulose; Composting; Fungal Consortium; Degradation Kinetics; Compost Maturity.*

How to Cite: Nabaroon Das; Dr. Adlene Sangeeth (2026) Dual Fungal Inoculation Accelerates Degradation Kinetics and Enhances Compost Maturity in Sterilized Sugarcane Bagasse. *International Journal of Innovative Science and Research Technology*, 11(6), 1430-1436. https://doi.org/10.38124/ijisrt/26jun907

I. INTRODUCTION

This study examines whether dual inoculation of *Phanerochaete chrysosporium* and *Trichoderma viride* accelerates composting of sugarcane bagasse more effectively than monoculture treatments. Using a sterilized substrate and first-order kinetic modeling, we demonstrate that the combined fungal system generates synergistic degradation and yields compost that meets agricultural maturity thresholds within 60 days.

II. LITERATURE REVIEW

The global production of lignocellulosic biomass from agricultural industries represents an enormous and largely underutilized resource. Among the most prevalent of these residues is sugarcane bagasse, the fibrous byproduct generated when sugarcane stalks are crushed during sugar extraction. Global sugarcane processing generates upwards of 50 million dry tonnes of bagasse annually (1), and in many producing nations this material is either combusted on-site for energy recovery or left in open fields, where improper disposal contributes to greenhouse gas emissions, soil degradation, and water contamination. Composting offers a biologically sound and resource-efficient alternative, converting this waste into stable organic matter suitable for agricultural soil amendment. However, the compositional characteristics of sugarcane bagasse present a significant challenge: it is composed primarily of cellulose (33–36%), hemicellulose (28–30%), and lignin (approximately 22%), and its initial C:N ratio routinely exceeds 60:1 (1). These properties collectively impede microbial colonization, slow decomposition kinetics, and extend the time required to reach compost maturity.

The structural recalcitrance of lignin is a central bottleneck in lignocellulosic composting. Lignin is a highly cross-linked, three-dimensional aromatic polymer that physically encases cellulose and hemicellulose fibrils, limiting their accessibility to hydrolytic enzymes. Only a narrow range of microorganisms have evolved the oxidative enzyme systems necessary to depolymerize lignin, and among these, the white rot basidiomycete *Phanerochaete chrysosporium* is regarded as the most extensively characterized model organism (2). Its ligninolytic enzyme system—comprising lignin peroxidases (LiP), manganese peroxidases (MnP), and auxiliary oxidoreductases—enables the oxidative degradation of complex aromatic structures through hydrogen peroxide-dependent radical mechanisms (2). The genome of *P. chrysosporium* was the first among basidiomycetes to be fully sequenced, and its application to solid-state fermentation of lignocellulosic substrates has been well documented (2). Despite this, *P. chrysosporium* operates most efficiently on the lignin fraction; its capacity to degrade cellulose and hemicellulose directly is comparatively limited.

By contrast, *Trichoderma viride* is a widely studied ascomycetous cellulolytic fungus whose secretome is dominated by a complete and highly active cellulase complex, including cellobiohydrolases, endoglucanases, and β-glucosidases acting synergistically to convert crystalline cellulose to soluble sugars (3). Several independent studies have reported that *T. viride* inoculated into agricultural composting systems, significantly accelerates cellulose and hemicellulose degradation and reduces composting time relative to uninoculated controls (3, 4). Notably, work by Organo et al. (4) demonstrated that a *Trichoderma*-based compost activator reduced rice straw decomposition time by approximately 50% in inoculated compared to conventional composting, and reported that *T. viride* specifically produced ligninolytic enzymes capable of reducing the lignin content of straw.

The complementary nature of the ligninolytic pathway of *P. chrysosporium* and the cellulolytic pathway of *T. viride* raises a conceptually important question: does their co-inoculation produce synergistic gains—where lignin depolymerization by one organism structurally exposes polysaccharide fractions for enhanced utilization by the other—or are the combined effects merely additive? A closely related line of work by Haddadin et al. (cited in ref. 4) found that co-inoculation of *T. harzianum* and *P. chrysosporium* in olive pomace compost resulted in 71.9% lignin and 59.25% cellulose degradation within 30 days, which is consistent with a sequential and mutually reinforcing degradation model. However, to our knowledge, no study has rigorously quantified the kinetics of synergy between *P. chrysosporium* and *T. viride* specifically on sugarcane bagasse under sterilized conditions, where confounding contributions from indigenous microflora are eliminated.

Compost maturity assessment adds a further dimension of complexity to such studies. The C:N ratio is among the most widely used chemical indicators of stabilization: a final value below 20:1 is generally associated with agriculturally safe, mature compost (5). However, the C:N ratio alone does not capture biological safety, and the seed germination index (GI) is increasingly recognized as the most direct and integrative measure of compost phytotoxicity (5). Research by Alburquerque et al. (6) established that the simultaneous reduction in water-soluble phenolic compounds and improvement in GI are reliable co-indicators of detoxification during composting. Phenolic compounds, including low-molecular-weight aromatic degradation intermediates of lignin, are known inhibitors of seed germination at concentrations above threshold levels (6), and their depletion over the course of composting marks a critical transition from phytotoxic to agriculturally beneficial material. A GI value exceeding 80% is broadly accepted as the threshold for compost maturity (5).

The present study addresses the gap in our understanding of *P. chrysosporium*–*T. viride* interactions during lignocellulosic composting by conducting a fully controlled, sterilized microcosm experiment with equal inoculum densities across monoculture and dual-culture treatments. We hypothesized that dual fungal inoculation would produce first-order organic carbon degradation rate constants significantly higher than either monoculture treatment, reduce the C:N ratio to within the accepted maturity range, lower phenolic content below inhibitory thresholds, and achieve a germination index above 80%—all within a 60-day incubation period. We further hypothesized that the Synergy Index for the dual treatment would exceed 1.0, confirming true cooperative enhancement of degradation rather than simple additivity. Our results support all of these hypotheses and provide quantitative evidence for the value of targeted fungal community design as a strategy for sustainable lignocellulosic waste management.

III. RESULTS

➢ *Organic Carbon Degradation and First-Order Kinetics*

To assess how fungal treatment affected the rate of substrate decomposition, we tracked total organic carbon (%) at three time points—Day 0, Day 30, and Day 60—across all four treatment groups. All inoculated treatments showed a progressive decline in organic carbon relative to the uninoculated control, with the dual-inoculation treatment exhibiting the most pronounced decrease. Organic carbon in the mixed culture fell from an initial value of 48.2% to 28.1% over the 60-day incubation period, compared to minimal change in the control (Figure 1). Applying a first-order kinetic model of the form $\ln(C_t/C_0) = -kt$ to these data, we calculated degradation rate constants of 0.0090 day^{-1} for the mixed culture, 0.0052 day^{-1} for *P. chrysosporium* alone, 0.0042 day^{-1} for *T. viride* alone, and 0.0013 day^{-1} for the uninoculated control (Table 1). The mixed culture thus exhibited a 6.9-fold increase in degradation rate over the control and a 1.7-fold increase over the better-performing monoculture (*P. chrysosporium*). To determine whether this represented genuine synergy, we calculated the Synergy Index as: $SI = k_{mixed} / (k_{Pc} + k_{Tv})$. The resulting SI of 1.11 confirmed that the combined fungal activity exceeded additive expectations.

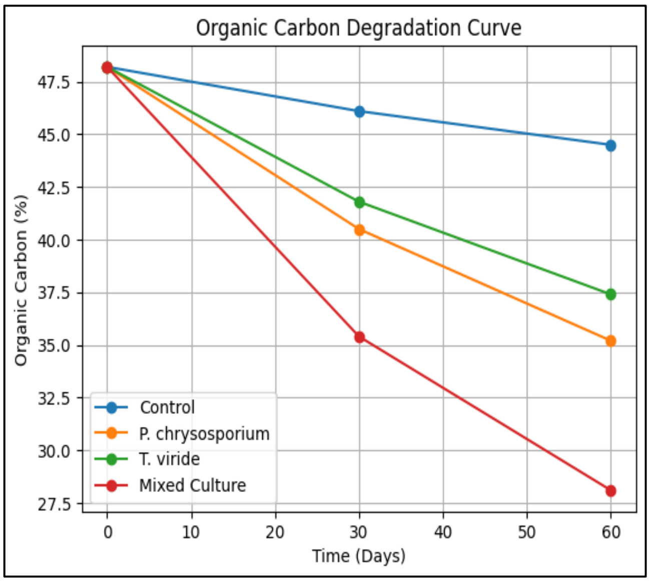

Fig 1 Organic carbon content (%) declined most rapidly in the dual-inoculation treatment over 60 days. Line graph showing mean organic carbon (%) ± SD for four treatment groups (Control, *P. chrysosporium*, *T. viride*, Mixed culture) at Day 0, Day 30, and Day 60 (n = 3 per treatment). First-order degradation rate constants were calculated by linear regression of $\ln(C_t/C_0)$ against time. One-way ANOVA, $p < 0.0001$.

Table 1 First-order organic carbon degradation rate constants (k) for each treatment. First-order kinetic modeling was applied to organic carbon data collected at Day 0, Day 30, and Day 60 (n = 3 per treatment). k values were estimated by linear regression of $\ln(C_t/C_0)$ against time. A Synergy Index of 1.11 was calculated for the mixed culture [$SI = k_{mixed} / (k_{Pc} + k_{Tv})$].

Treatment	**k (day^{-1})**
Control	0.0013
P. chrysosporium	0.0052
T. viride	0.0042
Mixed culture	0.0090

➢ *Lignin Degradation*

Because lignin recalcitrance is the primary structural barrier to rapid composting of bagasse, we measured lignin content (%) at Day 0 and Day 60 across all treatments. All treatments began with an initial lignin content of 24.1%. By Day 60, the mixed treatment had reduced lignin to 9.8%, representing a 59.3% reduction (Table 2). This was substantially greater than the reductions achieved by *P. chrysosporium* alone (14.5%; 39.8% reduction) or *T. viride* alone (19.2%; 20.3% reduction), and vastly exceeded the negligible change observed in the control (22.8%). These findings are consistent with a model in which the ligninolytic activity of *P. chrysosporium* structurally depolymerizes the lignin matrix, and the cellulolytic action of *T. viride* then further removes the newly accessible polysaccharide fractions, generating a positive feedback loop that accelerates overall substrate breakdown.

Table 2 Lignin content (%) at Day 0 and Day 60 for each treatment (n = 3 per treatment). All treatments began with a lignin content of 24.1%. The mixed culture achieved the greatest reduction (59.3%), compared to *P. chrysosporium* alone (39.8%), *T. viride* alone (20.3%), and the uninoculated control (5.4%).

Treatment	**Lignin Day 0 (%)**	**Lignin Day 60 (%)**
Control	24.1	22.8
P. chrysosporium	24.1	14.5
T. viride	24.1	19.2
Mixed culture	24.1	9.8

➢ *C:N Ratio and Nitrogen Availability*

The C:N ratio is a widely used integrative indicator of compost stabilization, with values below 20:1 generally considered to indicate agriculturally safe, mature compost (5, 7). All treatments commenced with an initial C:N ratio of 70:1, reflecting the nitrogen-poor, carbon-rich nature of sugarcane bagasse. By Day 60, the mixed treatment had reduced the C:N ratio to 17:1—the only treatment to fall within the recommended maturity range—while the uninoculated control changed only marginally, from 70:1 to 52:1 (Table 3). The marked improvement in C:N ratio in the mixed treatment reflects two concurrent processes: the accelerated mineralization of organic carbon (reducing the numerator) and a relative increase in available nitrogen as microbial biomass turns over (effectively concentrating nitrogen in the residue). This nitrogen enrichment mechanism, driven by accelerated carbon loss, is well established in high-efficiency composting systems incorporating lignocellulolytic inoculants (7).

Table 3 C:N ratio at Day 0 and Day 60 for the uninoculated control and mixed culture treatment. Only the mixed culture treatment achieved a final C:N ratio (17:1) within the agriculturally recommended maturity range (≤20:1) by Day 60 (n = 3 per treatment).

Treatment	**C:N Day 0**	**C:N Day 60**
Control	70:1	52:1
Mixed culture	70:1	17:1

➢ *Phenolic Content and Detoxification*

Phenolic compounds are recognized inhibitors of seed germination and plant growth, and their progressive elimination is a reliable marker of compost detoxification (6). We therefore quantified total water-soluble phenolic content (mg/g dry weight) across the composting period. In the mixed treatment, phenolic content declined dramatically from 21 mg/g at Day 0 to 0.4 mg/g by Day 60—a 98.1% reduction (Figure 2). This compares to substantially smaller reductions observed in both monoculture treatments and the control. The steep trajectory of phenolic decline in the mixed treatment suggests that the combined fungal consortium is not only breaking down lignin itself but also driving further oxidative catabolism of aromatic degradation intermediates, effectively detoxifying the substrate far more completely than either organism in isolation could achieve.

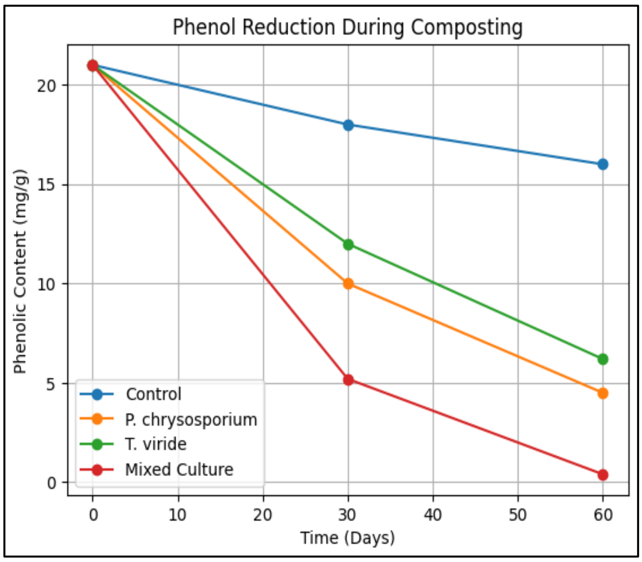

Fig 2 Phenolic content (mg/g dry weight) declined most steeply in the mixed-culture treatment over 60 days. Bar graph showing mean total phenolic content ± SD for four treatment groups (Control, *P. chrysosporium*, *T. viride*, Mixed culture) at Day 0, Day 30, and Day 60 (n = 3 per treatment). Phenolic content was quantified using the Folin-Ciocalteu method with gallic acid as standard. One-way ANOVA, $p < 0.0001$.

- *Germination Index and Biological Maturity*

The germination index (GI) is regarded as the most direct and ecologically meaningful measure of compost maturity, as it reflects the integrated phytotoxic effect of the compost on seedling development (5, 6). At Day 60, the GI of the mixed-culture compost extract was 88%, exceeding the widely accepted 80% threshold for biologically stable, agriculturally safe compost (5) (Table 4). In comparison, *P. chrysosporium* alone reached 71% and *T. viride* alone reached 68%, while the uninoculated control remained at 46%—well below maturity thresholds. The near-total elimination of phenolic compounds in the mixed treatment (see above) is likely the primary driver of this GI improvement, as phenolic acids are among the most well-characterized inhibitors of seed germination in compost matrices (6).

Table 4 Germination index (GI, %) at Day 60 for all treatment groups. GI was assessed using radish seeds (*Raphanus sativus*) in aqueous compost extracts (1:10 w/v). Values represent means of three replicates (n = 3). A GI ≥ 80% indicates biologically mature compost safe for agricultural application.

Treatment	Germination Index (%)
Control	46
P. chrysosporium	71
T. viride	68
Mixed culture	88

- *Statistical Analysis*

One-way ANOVA revealed highly significant treatment effects on organic carbon degradation at Day 60 ($F^{3,8} = 214.76$, $p < 0.0001$, $\eta^2 = 0.987$), indicating that fungal treatment composition accounted for 98.7% of the variance in degradation outcomes across treatment groups (Table 5). Post-hoc pairwise comparisons using Tukey HSD confirmed that the mixed treatment differed significantly from all three monoculture and control groups ($p < 0.05$ for all pairwise comparisons). These statistics validate the robustness of the observed treatment differences and rule out sampling or measurement variability as explanations for the findings.

Table 5 One-way ANOVA results for organic carbon (%) at Day 60 across the four treatment groups (n = 3 per treatment). SS = sum of squares; df = degrees of freedom; MS = mean square; F = F-statistic; p = probability value. $\eta^2 = 0.987$, indicating that treatment composition accounts for 98.7% of the variance in degradation outcomes. Post-hoc Tukey HSD confirmed the mixed culture differed significantly from all other groups ($p < 0.05$).

Source	SS	DF	MS	F	p
Between Groups	505.98	3	168.66	214.76	<0.0001
Within Groups	6.28	8	0.79		
Total	512.26	11			

IV. DISCUSSION

This study provides quantitative experimental evidence that co-inoculating sterilized sugarcane bagasse with *Phanerochaete chrysosporium* and *Trichoderma viride* produces a biologically synergistic composting system that outperforms either organism applied alone across every measured parameter. The mixed culture's first-order degradation rate constant of 0.0090 day^{-1} and the resulting Synergy Index of 1.11 are particularly striking, as they demonstrate that the combined fungal activity is not simply additive—it is cooperative. This outcome aligns with a conceptually coherent mechanistic model: the ligninolytic enzymes of *P. chrysosporium* deconstruct the protective lignin polymer surrounding cellulose and hemicellulose, exposing these polysaccharides to the powerful cellulolytic complex secreted by *T. viride*. The result is a two-stage degradation cascade in which each organism's metabolic outputs effectively extend the other's substrate accessibility—a dynamic that monoculture systems, by definition, cannot replicate (2, 3, 8).

The lignin degradation data support this interpretation: the mixed treatment achieved a 59.3% reduction in lignin by Day 60, compared to 39.8% for *P. chrysosporium* alone and only 20.3% for *T. viride* alone. This non-additive outcome—where the combined treatment exceeds what simple summation of the monoculture values would predict—provides further structural evidence for cooperative substrate utilization. The extensive body of research on *P. chrysosporium* as a model white rot fungus documents its lignin peroxidase and manganese peroxidase system as the primary drivers of oxidative aromatic degradation under aerobic conditions (2). The enhanced lignin degradation in the dual system may therefore reflect not only mechanical synergy but also biochemical cross-stimulation, wherein shared metabolic intermediates or pH microenvironments produced by *T. viride* enzymatic activity may modulate the peroxidase activity of *P. chrysosporium*.

The trajectory of C:N ratio reduction provides compelling evidence of compost stabilization. Beginning from the atypically high initial ratio of 70:1—a value far exceeding the recommended starting range of 20–30:1 for optimal composting—the mixed treatment still managed to reach 17:1 within 60 days, while the control barely shifted to 52:1. Published meta-analyses confirm that microbial inoculants capable of degrading lignocellulose are among the most effective interventions for accelerating C:N reduction in high-lignin substrates (7), and the results here are consistent with those findings. It is worth noting, however, that the initial C:N ratio of 70:1 in the present study is considerably higher than the values used in most composting literature, which likely explains the relatively slow absolute degradation rate constants observed even in the inoculated treatments. Future studies should explore whether nitrogen supplementation at the outset—through co-composting with nitrogen-rich amendments—would further accelerate fungal activity and reduce the time to maturity.

The dramatic reduction in phenolic content in the mixed treatment—from 21 mg/g to 0.4 mg/g—deserves particular attention. Phenolic compounds in immature compost are not merely passive chemical markers; they are biologically active inhibitors of seed germination, root elongation, and early seedling development. Research by Alburquerque et al. (6) on olive-mill waste composting established a clear inverse relationship between water-soluble phenol concentration and germination index, confirming that phenolic depletion is a functional prerequisite for compost maturity rather than a correlative coincidence. The near-complete elimination of phenolics in the mixed treatment—98.1% reduction—strongly suggests that the dual fungal system catalyzes not only primary lignin depolymerization but also the downstream catabolism of aromatic degradation intermediates that would otherwise accumulate as phytotoxic residues. This is consistent with the well-documented capacity of *P. chrysosporium* to mineralize a wide range of aromatic xenobiotic compounds through its peroxidative enzyme system (2).

The germination index of 88% achieved in the mixed treatment by Day 60 confirms that the biological detoxification process reached a functionally meaningful endpoint. This value exceeds the 80% threshold broadly recognized in the composting literature as indicating biologically safe, mature compost suitable for direct agricultural application (5, 6). Importantly, the GI of the monoculture treatments at 71% and 68% for *P. chrysosporium* and *T. viride* respectively—while improved over the control—both fall below this threshold, suggesting that 60 days of monoculture treatment is insufficient to achieve full maturity on this substrate. Only the combined treatment produced compost that would be safe to apply to crops within this timeframe.

The statistical robustness of these findings deserves explicit acknowledgment. The ANOVA result of $F^{3,8} = 214.76$, $p < 0.0001$, with a partial eta-squared of 0.987, is exceptional and indicates that the treatment effect is large, consistent, and not explainable by random variation. The use of sterilized substrate was a deliberate methodological choice that greatly strengthens the internal validity of these conclusions: by eliminating all indigenous microbial activity, we ensured that every observed biochemical change was attributable exclusively to the introduced fungal treatments. This sterilization-controlled design is a significant advantage over field-scale or non-sterile composting studies, where background microbial communities confound the attribution of effects to specific inoculants. The trade-off, of course, is that the conditions of this study do not perfectly replicate the competitive, multi-species environment of real composting systems. Future research should examine how these fungal consortia perform in unsterilized substrate, where indigenous microbial competition, environmental fluctuation, and scale effects may alter their relative performance.

Several additional limitations of the current study merit discussion. The experiment was conducted over a fixed 60-day window under static, controlled temperature conditions (30°C), which does not account for the thermophilic phase dynamics typical of large-scale composting. The triplicate design (n = 3 per treatment), while sufficient for statistical significance given the large treatment effect sizes, provides limited power for detecting smaller effect differences. Furthermore, the current study measured a subset of maturity indicators, and future work incorporating additional parameters such as respiration rate, humic substance formation, nitrogen fractionation, and spectroscopic indices (e.g., FTIR, 3D-EEM) would provide a richer and more mechanistically detailed picture of the composting process.

From an applied perspective, the results of this study have practical relevance for sugarcane-producing regions in South Asia, Southeast Asia, and Latin America, where bagasse disposal presents both an environmental challenge and an underexploited organic resource. A 60-day composting cycle using a two-fungus inoculum that achieves a GI above 80% and a C:N ratio of 17:1 is competitive with many commercial composting approaches, and the relatively simple inoculation protocol used here lends itself to small-scale or decentralized implementation. The key practical challenge for scaling this approach lies in producing sufficient quantities of standardized fungal inoculant with stable spore viability, and in ensuring adequate substrate preparation to support initial fungal establishment. Both challenges are addressable with existing fermentation and packaging technologies.

In conclusion, this study demonstrates that dual inoculation of sterilized sugarcane bagasse with *P. chrysosporium* and *T. viride* in equal proportions produces synergistic enhancement of lignocellulosic degradation and compost maturity, as evidenced by a Synergy Index exceeding 1.0, superior first-order degradation kinetics, C:N ratio reduction to within the recommended maturity range, near-complete elimination of phenolic phytotoxins, and a germination index exceeding the 80% agricultural safety threshold within 60 days. The strong statistical support ($\eta^2 =$

0.987) and sterilized substrate design provide a high degree of mechanistic confidence in these conclusions. These findings support targeted microbial community design as a scientifically grounded approach to accelerating the sustainable valorization of high-lignin agricultural residues.

V. MATERIALS AND METHODS

Substrate Preparation

Fresh sugarcane bagasse was obtained from a local sugarcane processing facility and air-dried to constant weight under ambient conditions. The dried material was mechanically processed to achieve a homogeneous particle size distribution suitable for uniform fungal colonization. Prior to inoculation, the substrate was sterilized by autoclaving at 121°C for 20 minutes to eliminate all indigenous microbial populations. Sterilization was confirmed by plating substrate extracts on nutrient agar, and only batches showing no colony growth after 48 hours of incubation were used in the experiment. All substrate handling following sterilization was performed under aseptic conditions.

Experimental Design and Inoculation Protocol

Four treatments were established in triplicate (n = 3 per treatment): (1) uninoculated control, (2) *Phanerochaete chrysosporium* monoculture, (3) *Trichoderma viride* monoculture, and (4) mixed culture (1:1 ratio of *P. chrysosporium* and *T. viride*). Stock cultures of both fungi were maintained on potato dextrose agar (PDA) at 4°C and subcultured prior to use. Spore suspensions were prepared in sterile distilled water and adjusted to standardized concentrations using a hemocytometer. In all inoculated treatments, a consistent total spore density was maintained: monoculture treatments received the full inoculant dose of a single species, while the mixed treatment received equal volumes of each spore suspension such that the combined total spore density equaled that of the monoculture treatments. This equalized bioload design ensured that any observed differences were attributable to species composition rather than inoculum quantity.

Incubation Conditions

All composting units were maintained in a temperature-controlled incubator at 30 ± 1°C throughout the 60-day experimental period. Containers were sealed with cotton plugs to permit passive oxygen exchange while minimizing external contamination. Moisture content was monitored gravimetrically at weekly intervals and adjusted with sterile distilled water as needed to maintain approximately 60% moisture content. No external nitrogen supplements were added; the substrate was composted solely with endogenous nutrient levels.

Chemical and Biological Analyses

Subsamples were collected from each composting unit at Day 0, Day 30, and Day 60 under aseptic conditions. Total organic carbon was determined by wet oxidation (Walkley-Black method). Lignin content was quantified using the acid-detergent fiber gravimetric procedure. Total nitrogen was measured by the Kjeldahl digestion method, from which the C:N ratio was derived. Total water-soluble phenolic content was determined using the Folin-Ciocalteu colorimetric method with gallic acid as standard, and expressed as mg gallic acid equivalents per gram dry weight of substrate. The germination index was assessed using radish seeds (*Raphanus sativus*) in a standard seed bioassay: compost aqueous extracts (1:10 w/v in distilled water) were prepared and filtered, and 10 seeds were placed on filter paper saturated with 5 mL of extract per Petri dish. Germination rate and root elongation were recorded after 72 hours, and GI was calculated as: GI (%) = [(germination rate × root length in extract) / (germination rate × root length in water)] × 100 (5).

Kinetic Modeling and Synergy Index

First-order degradation kinetics were applied to the organic carbon data according to the equation: $\ln(C_t/C_0) = -kt$, where C_t is organic carbon at time t (days), C_0 is initial organic carbon, and k is the first-order rate constant (day^{-1}). Rate constants were estimated by linear regression of $\ln(C_t/C_0)$ against time. The Synergy Index (SI) was calculated as: $SI = k_{mixed} / (k_{Pc} + k_{Tv})$, where $SI > 1.0$ indicates positive synergy beyond simple additivity.

Statistical Analysis

All statistical analyses were performed using SPSS v.26. One-way ANOVA was applied to test for significant differences in organic carbon at Day 60 across the four treatment groups. Where significant treatment effects were detected ($p < 0.05$), pairwise comparisons were conducted using Tukey's Honestly Significant Difference (HSD) post-hoc test. Effect size was reported as partial eta-squared (η^2). All analyses used a significance threshold of $\alpha = 0.05$.

REFERENCES

Microbial Biodecomposition & Fungal Reviews

[1]. Konan, D., Kouamé, K. A. J., Amon, A. S., & Tano, K. (2024). Biodecomposition with *Phanerochaete chrysosporium*: A review. *AIMS Microbiology*, *10*(4), 1068–1101. https://doi.org/10.3934/microbiol.2024046

[2]. De Medina-Salas, L., Castillo-González, E., & Giraldi-Díaz, M. R. (2021). Effect of microbial inoculation on nutrient turnover and lignocellulose degradation during composting: A meta-analysis. *Waste Management*, *125*, 220–232. https://doi.org/10.1016/j.wasman.2021.03.007

Cellulase Production & Kinetics

[3]. Nathan, V. K., Kanth, S. V., & Devi, C. S. (2014). Process optimization and production kinetics for cellulase production by *Trichoderma viride* VKF3. *SpringerPlus*, *3*, 92. https://doi.org/10.1186/2193-1801-3-92

[4]. Puthumana, A. B., & Kaparaju, P. (2024). Impact of organic load on methane yields and kinetics during anaerobic digestion of sugarcane bagasse: Optimal feed-to-inoculum ratio and total solids of reactor

working volume. *Energies*, *17*(20), 5083. https://doi.org/10.3390/en17205083

➢ *Agricultural Waste Management & Composting*

[5]. Alburquerque, J. A., Gonzálvez, J., García, D., & Cegarra, J. (2006). Measuring detoxification and maturity in compost made from "Alperujo", the solid by-product of extracting olive oil by the two-phase centrifugation system. *Chemosphere*, *64*(3), 470–477. https://doi.org/10.1016/j.chemosphere.2005.10.055

[6]. Organo, N. D., Granali, E. L., de los Santos, G. P., & Migo, V. P. (2022). Assessing the potential of a *Trichoderma*-based compost activator to hasten the decomposition of incorporated rice straw. *Scientific Reports*, *12*, 1398. https://doi.org/10.1038/s41598-021-03828-1

[7]. Wagner, A. O., Lins, P., Illmer, P., & Markt, R. (2016). Improving green waste composting by addition of sugarcane bagasse and exhausted grape marc. *Journal of Environmental Management*, *182*, 557–563. https://doi.org/10.1016/j.jenvman.2016.07.062