

Enhancing Microbial and Enzyme Activity: The Effect of Vermicomposting with *Eudrilus eugeniae* Using Cow Dung , Sugarcane bagasse and *Gliricidia sepium*

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Abstract: - Understanding the interplay between earthworms, microbes, and enzymes is crucial for assessing soil functionality and health. This study investigates the activities of microbes and enzymes in vermicompost produced by *Eudrilus eugeniae*, using different ratios of sugarcane bagasse (SCB), *Gliricidia sepium* (GM), and cow dung (CD). Five substrate treatments were evaluated: VCT₁, VCT₂, VCT₃, VCT₄, and VCT₅. The initial microbial CFU and the final microbial CFU (worm-worked and worm-unworked) in the vermicombed substrates were Calculated to one-way ANOVA. Enzyme activities in vermicompost were analyzed using specific laboratory methods. The study spanned 50 days, with microbial populations and enzyme activities analyzed. Initial microbial counts increased significantly up to 30 days, then stabilized, with the highest levels observed in VCT₄. Enzyme activities, including dehydrogenase, urease, acid phosphatase, alkaline phosphatase, protease, and cellulase, initially increased but declined towards the end of vermicomposting. VCT₄ consistently showed the highest enzyme activities. The findings suggest that VCT₄ group of sugarcane bagasse, cow dung, and *Gliricidia sepium* is optimal for producing nutrient-rich vermicompost using *Eudrilus eugeniae*.

Keywords: *Eudrilus eugeniae*, microbes and enzymes, VCT₄, sugarcane bagasse, *Gliricidia sepium*.

I. INTRODUCTION

Since the 1960s' 'Green Revolution,' agrochemicals have been extensively utilized to increase food production (1). The excessive use of chemical fertilizers presents significant environmental challenges (2). While these fertilizers are instrumental in boosting crop yields to meet global food demand, their overuse can lead to a host of ecological issues, including soil degradation, waterway contamination, and disruption of local ecosystems (3). Nutrient runoff from overly fertilized fields contributes to water pollution problems such as eutrophication, which causes harmful algal blooms and dead zones in aquatic environments (4). Additionally, heavy reliance on chemical fertilizers can deplete soil health over time, reducing its organic matter and biodiversity, which are critical for sustainable agriculture. Therefore, it is imperative to adopt more sustainable practices, such as the use of organic fertilizers, to mitigate the adverse environmental impacts associated with high usage of chemical fertilizers (5). This approach not only preserves environmental health but also enhances food security for future generations (6). Among these, certain earthworm species enhance biological resistance through their casts (7,8). Due to the

adverse side effects associated with synthetic fertilizers and pesticides, researchers have increasingly turned their attention to organic alternatives such as vermicompost. These natural additions not only boost crop yields but also repel harmful pests without damaging the environment.

Vermicompost is the end product of the non-thermophilic biodegradation of organic materials, facilitated by interactions between earthworms and microorganisms (9,10). Key species involved include *Eudrillus enginea*, *Eisenia fetida*, *Lumbricus rubellus* and *Amyanthes diffrigens* (30,31). Within their intestines, earthworms host diverse microbes, enzymes, and hormones (29). As organic soil material passes through the earthworms' gizzard, it is converted into a fine aerosol. This material is further processed by intestinal microorganisms and enzymes, eventually excreted as a 'cast' (11). These 'casts' undergo additional treatment by gut-associated microbes to complete the vermicomposting process. Compared to natural soil, the casts produced by earthworms during vermicomposting can contain up to five times more nitrogen, seven times more phosphate, as well as higher levels of exchangeable calcium and soluble potassium (12). Additionally, these casts can harbor a thousand times more beneficial bacteria. Mounting scientific evidence indicates that vermicomposts significantly enhance plant growth and productivity (13).

Vermicompost (VC) is well-suited for mixing in substrates due to its beneficial physicochemical properties, including high porosity, excellent aeration, effective drainage, and substantial water-holding capacity (14). Furthermore, VC increases the surface area, providing additional micro-sites that facilitate the activity of decomposing microbes, thereby enhancing nutrient absorption and retention in plants. Consequently, vermicompost is recognized as an environmentally friendly approach to managing agricultural residues (15). Earthworms contribute to this process by improving the physical, chemical, and biological characteristics of the soil, which promotes plant growth and nutrient uptake (17).

Food processing wastes pose environmental challenges by consuming freshwater resources and occupying land (18,28). Sugarcane bagasse, the fibrous residue left after the extraction of juice from sugarcane, is a significant by-product of the sugar industry (18). While it is often regarded as a waste material, sugarcane bagasse has various uses that can either positively or negatively affect the environment, depending on how it is managed (19). Globally, 175.1 million metric tonnes of sugarcane are produced annually, with each tonne of processed sugarcane generating 280 kg of

sugarcane bagasse (20,21). Recycling SCB into organic fertilizer presents a safe and responsible method for its disposal, turning a waste product into a valuable resource (22).

Gliricidia sepium is known for its high levels of nitrogen, which is essential for composting processes (23). Its leaves decompose quickly, releasing nitrogen and other nutrients that are critical for enriching the vermicompost. The leaves of *Gliricidia sepium* are relatively soft and decompose faster than tougher, more fibrous plant residues (24). This quick decomposition aids in accelerating the vermicomposting process, making nutrients more readily available. The rapid breakdown of *Gliricidia sepium* stimulates microbial activity in the compost pile. These microbes are essential for the conversion of organic matter into stable compost and play a vital role in nutrient cycling within the compost (2021). *Gliricidia sepium* is an excellent choice for vermicomposting due to its nutrient-rich properties, fast decomposition rate, and beneficial effects on soil health and structure (26).

The combination of Green manure, sugarcane bagasse, and cowdung in vermicomposting could be intriguing due to their distinct characteristics. GM provides a tasty, easily biodegradable, but temporary food source for earthworms, whereas SCB serves as a permanent food source for sustaining earthworm populations. Additionally, contribute to the overall organic matter content. Furthermore, have been found to contain trace metals such as iron, nickel, and zinc, which are essential for microbial activity during fermentation (27). Studying the combined activity of earthworms, microbes, and enzymes provides a comprehensive understanding of soil functionality and health. This knowledge can lead to better management practices that promote sustainable agriculture, protect natural resources, and maintain ecological balance. Additionally, these studies contribute to the development of biotechnological applications such as biofertilizers and soil remediation techniques, which are crucial for enhancing crop productivity and environmental sustainability. The current investigation aims to examine the activities of microbes and enzymes in vermicompost produced by *Eudrilus eugeniae*, using different ratios of sugarcane bagasse, *Gliricidia sepium*, and cow dung.

II. MATERIALS AND METHODS

COLLECTION OF SAMPLES

The experiment was conducted at Annamalai University in Tamilnadu, India. For the study, the epigeic earthworm *Eudrilus eugeniae*, used in vermicomposting, was cultivated and collected from the Vermi Biotechnology Laboratory at the Department of Zoology, Annamalai University. Cow dung, chosen for biodegrading green manure and sugarcane bagasse, was sourced urine and straw-free from the dairy farm at the Faculty of Agriculture, Annamalai University. It was then sun-dried, powdered, and stored in jute bags. Sugarcane bagasse was obtained from the M.R.K Co-operative Sugar Mill in Sethiathope, cured for a month to eliminate any odor, and then utilized as bedding material. Additionally, the green leaves of *Gliricidia sepium* were collected around the campus of Annamalai University, Chidambaram.

EXPERIMENTAL DESIGN

Vermibeds were prepared using round tubs measuring 45 cm in height and 35 cm in diameter. Triplicate samples were established as part of the experimental setup, each containing different types of organic waste. Chopped organic waste was mixed with cow dung in various ratios. The vermibeds were maintained at room temperature, and the moisture content was kept at approximately 70-80% water. Each tub, containing the vermibed medium and substrate, was inoculated with 10 earthworms and an additional amount of cow dung. Five substrate treatments were developed by combining cow dung with sugarcane bagasse and green manure in different proportions.

MICROBIAL ACTIVITY

To analyze the microbiological populations during the composting and vermicomposting processes, the study involved several steps, specifically targeting different microbial groups such as total bacteria, fungi, actinomycetes. Here's a detailed breakdown of the procedure used for microbial analysis based on the Most Probable Number (MPN) method and colony forming units (CFU) as described in standards like APHA (2005). One gram of each sample was taken in a sterile conical flask containing nine ml of distilled water and shaken in a vortex mixer for 30 minutes. From this stock, various dilutions were prepared from 10^{-1} to 10^{-7} with sterile distilled water. One ml of the diluted sample was poured into Petri plates containing respective media for bacteria,

fungi and actinomycetes. Three replicates were maintained for each observation. The initial microbial CFU and the final microbial CFU (worm-worked and worm-unworked) in the vermibed substrates were calculated to one-way ANOVA.

ENZYME ACTIVITY

Samples were first air-dried, sieved to remove large particles, and stored in a cool, dry place. Cellulase Analysis: 1 g sample was incubated with 50 ml of 2% carboxymethyl cellulose (CMC) in a phosphate buffer at 50°C for 24 hours. Post-incubation, DNSA reagent was added, the mixture heated, and absorbance was measured at 540 nm. Phosphatase Analysis: 1 g sample was mixed with 100 ml of modified universal buffer containing p-nitrophenyl phosphate (pNPP) and incubated at 37°C for 1 hour. The reaction was stopped with NaOH, and the absorbance of the released p-nitrophenol was measured at 400 nm. Dehydrogenase Analysis: 5 g sample was incubated with triphenyl tetrazolium chloride (TTC) under anaerobic conditions at 37°C for 24 hours. The formed triphenylformazan was extracted using ethanol and its absorbance measured at 485 nm. Urease Analysis: 5 g sample was incubated with 50 ml of 10% urea solution at 37°C for 2 hours. Ammonia concentration was determined using Nessler's reagent with absorbance read at 425 nm. Protease Analysis: 1 g sample was mixed with 50 ml of 2% casein in phosphate buffer and incubated at 50°C for 1 hour. The reaction was halted with trichloroacetic acid (TCA), and absorbance of the supernatant was measured at 280 nm for tyrosine equivalents.

III. RESULTS

Dynamics of bacterial, fungal, and actinomycete populations during vermicomposting

The total microbial population in the initial vermibed substrates of all treatments increased over the vermicomposting period (Figs. 1 and 2). The microbial dynamics during vermicomposting of sugarcane bagasse and cow dung amended with *Gliricidia sepium* varied between treatments. Initial bacterial populations in treatments VCT₁, VCT₂, VCT₃, VCT₄, and VCT₅ were 40.42, 37.68, 41.79, 36.99, and 25.35 CFU × 10⁶ g⁻¹, respectively, rising to 65.24, 64.87, 60.88, 73.94, and 53.33 CFU × 10⁶ g⁻¹ after 50 days. Initial fungal populations were 16.44, 17.25, 17.16, 18.90, and 20.48 CFU × 10⁴ g⁻¹, rising to 37.03, 43.06, 38.70, 46.60, and 40.09 CFU × 10⁴ g⁻¹. Initial actinomycetes populations were 12.33, 15.18, 13.86, 16.10, and 14.08 CFU × 10³ g⁻¹, increasing to 37.37, 46.34,

42.70, 50.68, and 30.15 $\text{CFU} \times 10^3 \text{ g}^{-1}$. Bacterial population in VCT_5 (without cow dung) changed from 36.99 to 71.65 $\text{CFU} \times 10^6 \text{ g}^{-1}$ over 50 days. Fungal dynamics showed a similar trend with significant variation in final populations and Actinomycete populations in VCT_4 changed from 16.10 to 50.76 $\text{CFU} \times 10^4 \text{ g}^{-1}$ over 50 days.

Fig. 1: Variation in average microbial population between treatments during 50 days vermicomposting of sugarcane bagasse + cow dung amended with leafy green manure plant, *Gliricidia sepium* (50 days). (A) Bacteria, (B) Fungi and (C) Actinomycetes.

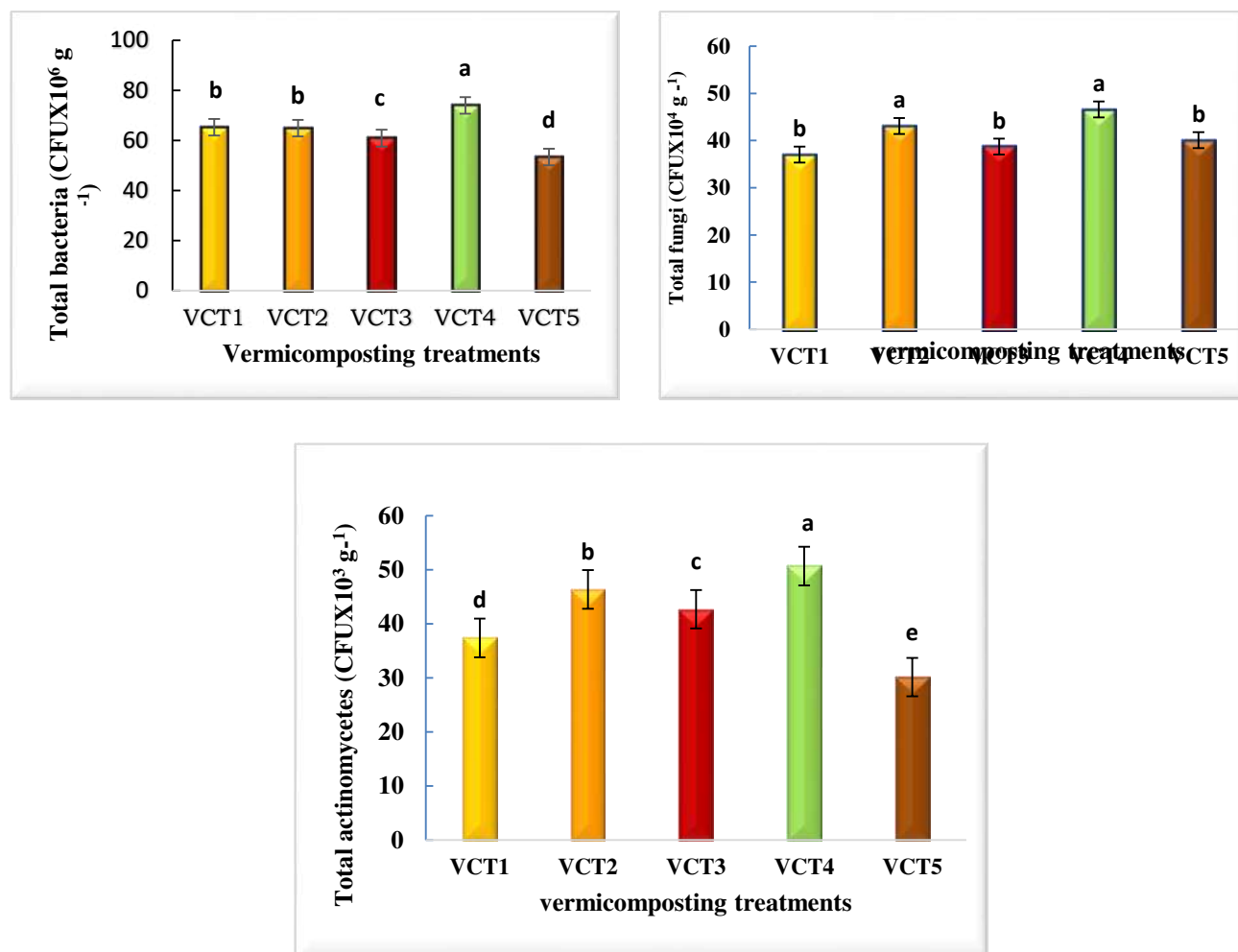
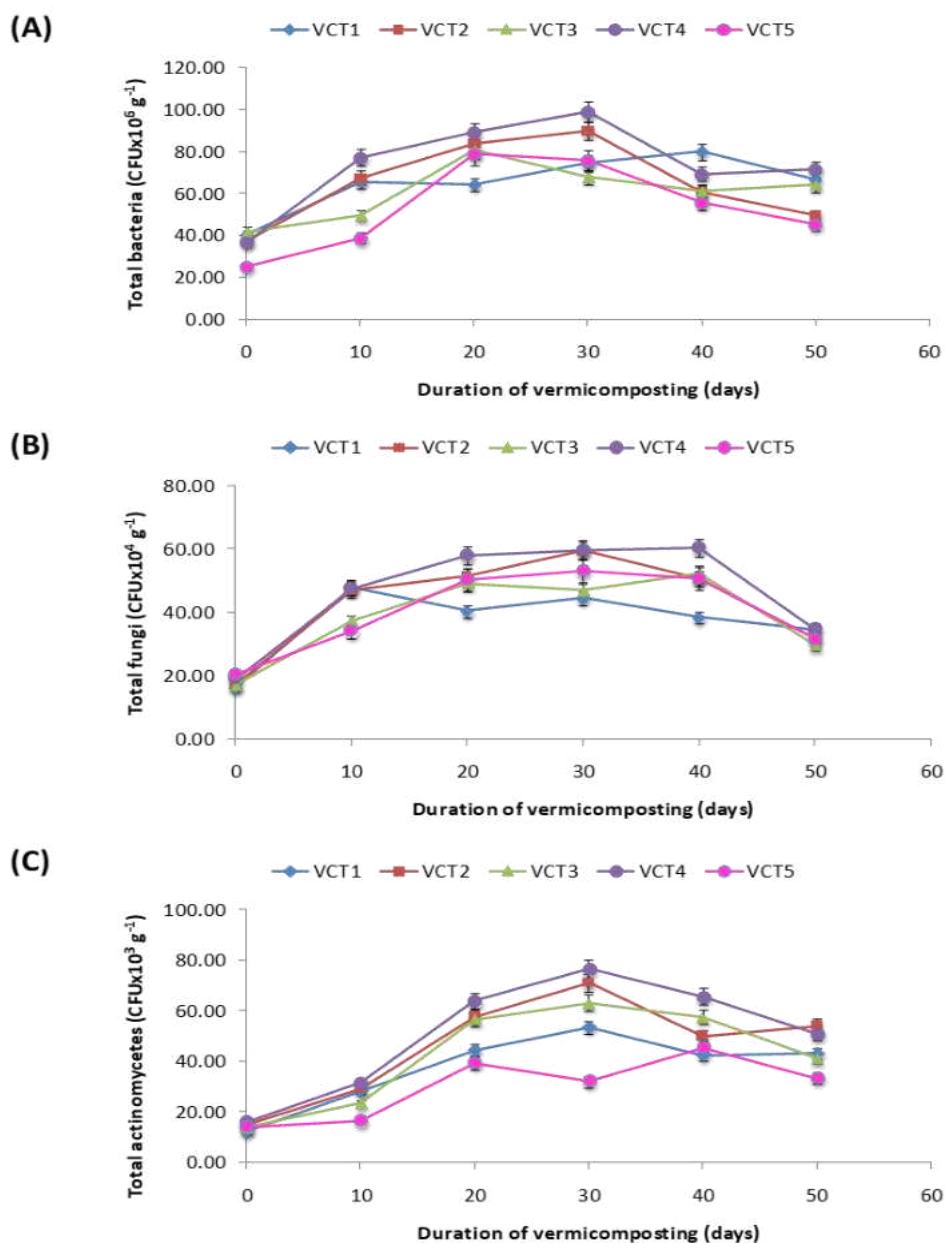


Fig. 2: Microbial population dynamics during vermicomposting of sugarcane bagasse + cowdung amended with leafy green manure plant, *Gliricidia sepium* (50 days). (A) Bacteria, (B) Fungi and (C) Actinomycetes.



Average activities and dynamics of dehydrogenase, urease, acid phosphatase, alkaline phosphatase, protease and cellulase during vermicomposting

The activities of enzymes (average over 50 days) and the dynamics of dehydrogenase, urease, acid phosphatase, alkaline phosphatase, protease, and cellulase are shown in Tables 1 and 2 and Figs. 3, 4, and 5. Generally, enzyme activities increased during the initial phases of vermicomposting of all sugarcane bagasse vermibed substrates, then decreased towards the end. There was variation in enzyme activities between treatments, with both significant and non-significant differences. The highest average dehydrogenase activity after 50 days was in VCT₄ ($45.06 \pm 2.87 \mu\text{g TPF g}^{-1} \text{ dw h}^{-1}$), significantly higher than other treatments (Table 1). Similar trends were observed for urease and acid phosphatase. Dehydrogenase dynamics in VCT₄ were 10.53, 16.68, 50.91, 68.46, 59.68, and $48.27 \mu\text{g TPF g}^{-1} \text{ dw h}^{-1}$ on days 0, 10, 20, 30, 40, and 50, respectively, and urease was 17.55, 26.33, 51.78, 67.58, 61.44, and $54.42 \text{ mg NH}_4^+ \text{ g}^{-1} \text{ dw h}^{-1}$ (Fig. 3).

Average activities of alkaline phosphatase ($72.56 \pm 5.22 \text{ mg PNP g}^{-1} \text{ dw h}^{-1}$), protease ($47.69 \pm 3.43 \text{ mg/g dw/24 h}$), and cellulase ($53.10 \pm 3.82 \text{ mg glucose/g/dw/h}$) were highest in VCT₄ and significantly different from other treatments ($P < 0.05$, Table 2). Enzyme activities typically peaked by day 30, then slowed. For example, alkaline phosphatase activity in VCT₁ was 17.17, 26.13, 48.53, 50.02, and $53.01 \text{ mg PNP g}^{-1} \text{ dw h}^{-1}$ on days 0, 10, 30, 40, and 50, respectively, with similar trends observed for other treatments and cellulase (Figs. 4 and 5).

Table : 1 Average Activities Of Enzyme, Dehydrogenase, Urease And Acid Phosphatase During The Period Of Vermicomposting Of Sugarcane Bagasse + Cow Dung Amended With Leafy Green Manure Plant, *Gliricidia Sepium* (50 Days).

Treatments	Dehydrogenase ($\mu\text{g TPF g}^{-1} \text{ dw h}^{-1}$)	Urease ($\text{mg NH}_4^+ \text{ g}^{-1} \text{ dw h}^{-1}$)	Acid Phosphatase ($\text{mg PNP g}^{-1} \text{ dw h}^{-1}$)
VCT ₁	30.36 ± 2.52^c	40.81 ± 2.63^c	47.16 ± 3.04^c
VCT ₂	32.07 ± 2.49^c	42.94 ± 2.71^{bc}	49.09 ± 3.09^{bc}
VCT ₃	38.15 ± 2.57^b	45.01 ± 2.93^b	51.46 ± 3.34^{ab}
VCT ₄	45.06 ± 2.87^a	47.98 ± 3.45^a	54.86 ± 3.95^a
VCT ₅	33.95 ± 2.94^{bc}	44.72 ± 3.33^b	51.13 ± 3.81^{ab}

Values are mean $\pm$ SEM; n = 3. Based on Tukey's HSD test, the different alphabets between treatments within each group are significantly different at $P < 0.05$.

Table: 2 Average Activities Of Enzyme, Alkaline Phosphatase, Protease And Cellulase During The Period Of Vermicomposting Of Sugarcane Bagasse + Cow Dung Amended With Leafy Green Manure Plant, *Gliricidia Sepium* (50 Days).

Treatments	Alkaline Phosphatase (mg PNP g ⁻¹ dw h ⁻¹)	Protease (mg/g dw/24 h)	Cellulase (mg glucose/g/dw/h)
VCT ₁	61.72 ± 3.98 ^c	40.57 ± 2.62 ^c	45.17 ± 2.91 ^c
VCT ₂	64.93 ± 4.09 ^c	42.68 ± 2.69 ^{bc}	47.52 ± 2.99 ^{bc}
VCT ₃	68.06 ± 4.42 ^b	44.73 ± 2.91 ^b	49.81 ± 3.24 ^{ab}
VCT ₄	72.56 ± 5.22 ^a	47.69 ± 3.43 ^a	53.10 ± 3.82 ^a
VCT ₅	67.62 ± 5.04 ^b	44.44 ± 3.31 ^b	49.49 ± 3.69 ^{ab}

Values are mean ± SEM; n = 3. Based on Tukey's HSD test, the different alphabets between treatments within each group are significantly different at P < 0.05.

Fig. 3: Dynamics of the enzyme activities, (A) dehydrogenase and (B) urease during vermicomposting of sugarcane bagasse + cow dung (1:1) amended with leafy green manure plant, *Gliricidia sepium* (50 days).

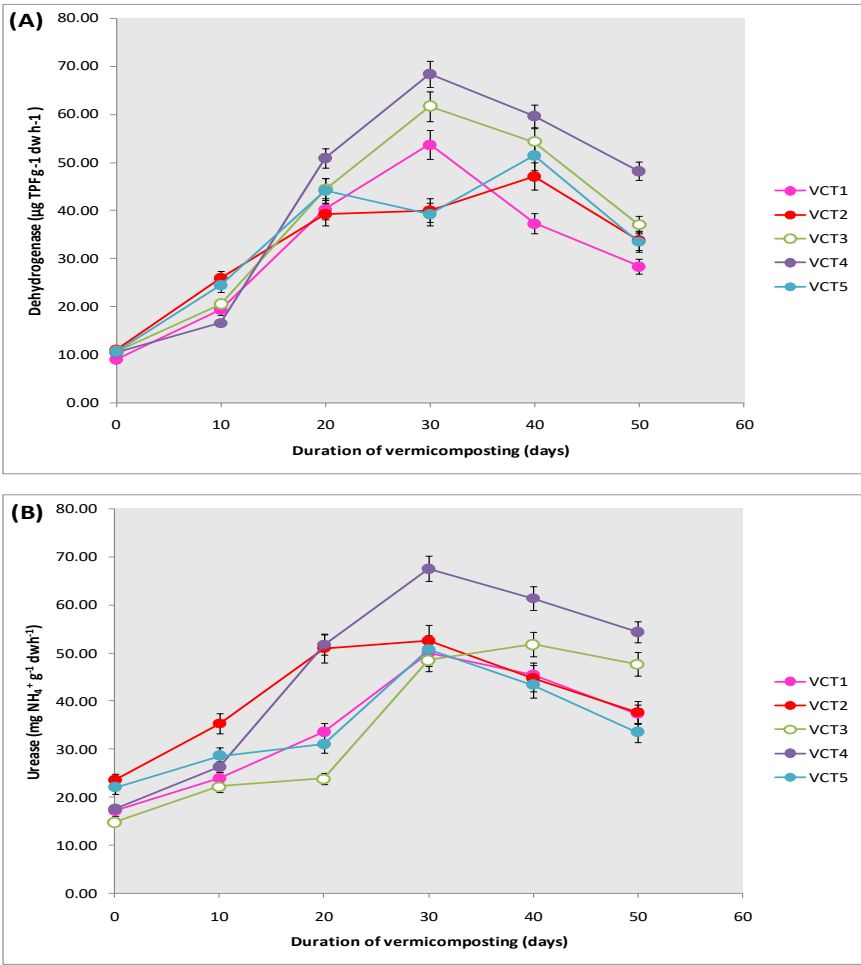


Fig. 4: Dynamics of the enzyme activities, (A) acid phosphatase and (B) alkaline phosphatase during vermicomposting of sugarcane bagasse + cow dung amended with leafy green manure plant, *Gliricidia sepium* (50 days).

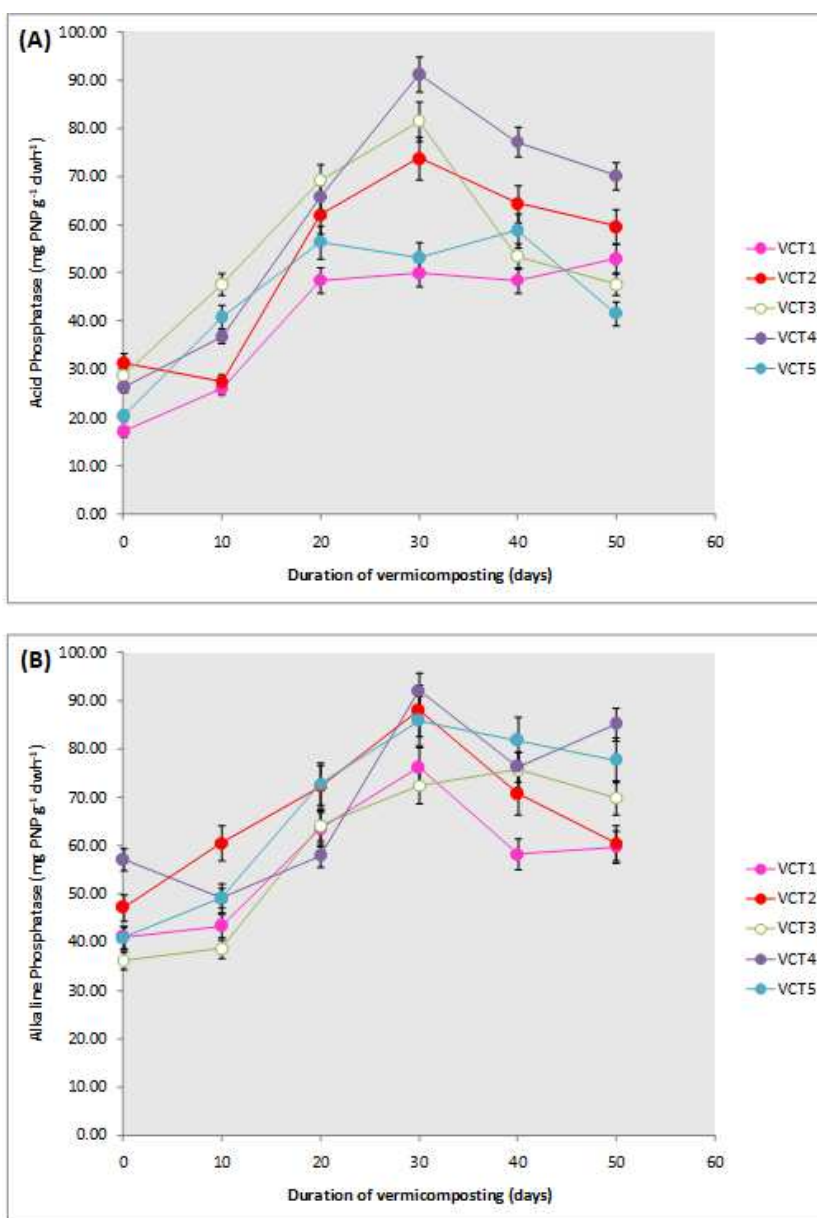
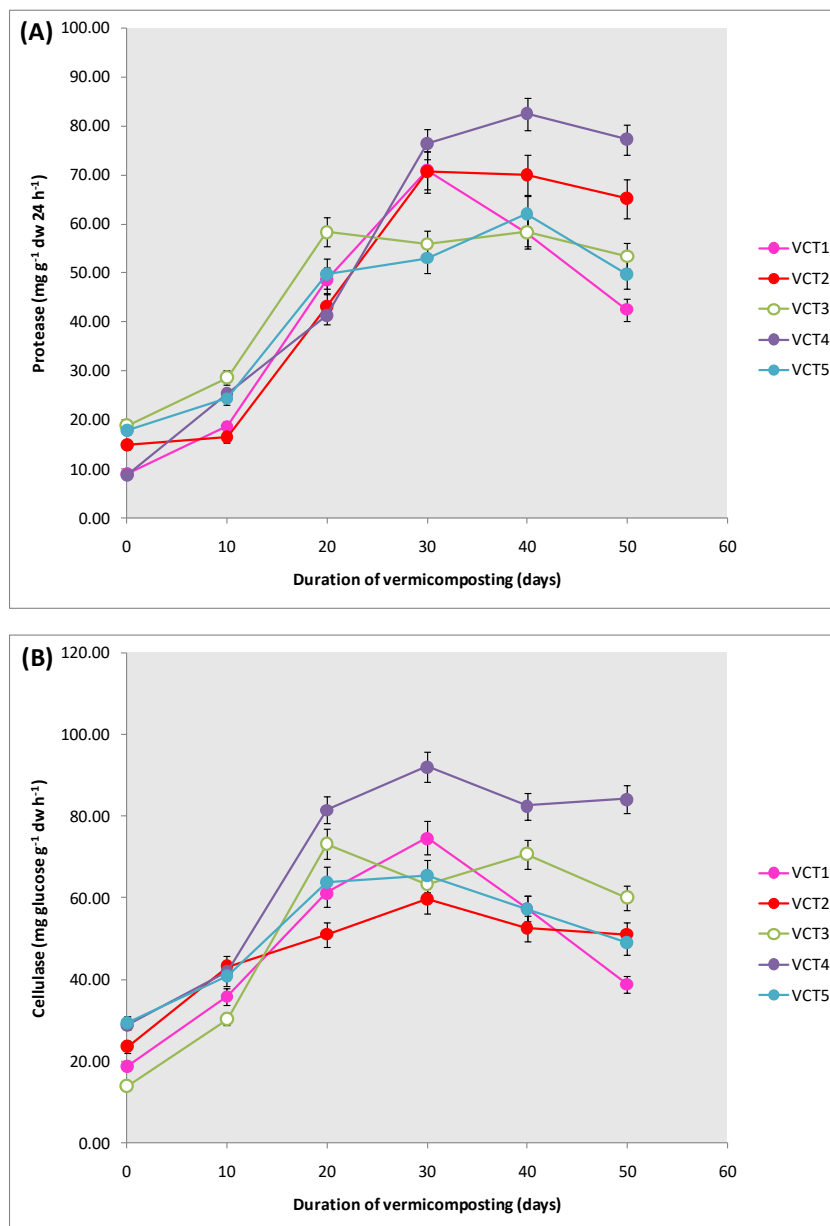


Fig. 5: Dynamics of the enzyme activities, (A) protease and (B) cellulase during vermicomposting of sugarcane bagasse + cow dung (1:1) amended with leafy green manure plant, *Gliricidia sepium* (50 days).



IV. DISCUSSION

Microbial activity is crucial during composting and vermicomposting for rapid mineralization and bioconversion of organic substrates into valuable composts. Microorganisms multiply quickly with suitable moisture and nutrients, secreting extracellular enzymes that break down organic materials, enhancing decomposition and compost formation (32,33).

In this study, the populations of bacteria, fungi, and actinomycetes increased significantly up to 30 days and then stabilized during the vermicomposting of sugarcane bagasse vermibed substrates. The initial bacterial populations in VCT₁, VCT₂, VCT₃, VCT₄, and VCT₅ were 40.42, 37.68, 41.79, 36.99, and 25.35 CFU $\times 10^6$ g⁻¹, respectively, rising to 65.24, 64.87, 60.88, 73.94, and 53.33 CFU $\times 10^6$ g⁻¹ after 50 days, with significant variation between treatments. Fungal dynamics showed a similar trend.

The actinomycetes population in VCT₄ was 16.10, 31.50, 63.65, 76.44, 65.60, and 50.76 CFU $\times 10^4$ g⁻¹ on days 0, 10, 20, 30, 40, and 50, respectively. Earlier studies have shown that earthworms' feeding habits impact soil ecosystem functioning by affecting the availability of organic detritus for soil microorganisms (34). The gut of earthworms acts as a 'natural bioreactor,' increasing the density of beneficial microbes in excreted materials (35).

Prakash and Karmegam (2010) recorded bacterial populations during vermicomposting as 51, 64, 99, 116, and 132 $\times 10^6$ CFU g⁻¹ on days 0, 15, 30, 45, and 60, respectively, with similar trends observed for fungi and actinomycetes during vermicomposting of *Ipomoea staphylinea* (Balachandar *et al.*, 2021). These findings corroborate the present study's results on microbial status.

The activity of extracellular enzymes in vermibed substrates indicates the status of organic substrate decomposition by earthworms and microorganisms. These degradative enzymes are mostly secreted by microbes, while earthworms secrete similar enzymes in their gut. Studying these enzymes and their dynamics provides an overall picture of the decomposition process in vermibed substrates.

In this study, the activities of dehydrogenase, urease, acid phosphatase, alkaline phosphatase, protease, and cellulase were analyzed at regular intervals during the vermicomposting of sugarcane bagasse. Generally, enzyme activities increased during the initial phases of vermicomposting and

then decreased towards the end. There were variations in enzyme activities between treatments, with some significant differences.

After 50 days, the average activity of dehydrogenase was highest in VCT₄ ($45.06 \pm 2.87 \mu\text{g TPF g}^{-1} \text{ dw h}^{-1}$), significantly higher than in other treatments. Similar trends were observed for urease and acid phosphatase. The average activities of alkaline phosphatase, protease, and cellulase in VCT₄ were 72.56 ± 5.22 , 47.69 ± 3.43 , and 53.10 ± 3.82 , respectively, and were significantly different from other treatments ($P < 0.05$). Enzyme activities generally peaked around 30 days and then slowed down. Alkaline phosphatase activity in VCT₁ showed a similar trend, as did cellulase activity.

Overall, enzyme activities were much higher in the VCT₄ vermibed combination (mixture of sugarcane bagasse, cow dung, and *Gliricidia sepium*) compared to VCT₁, VCT₂, VCT₃, and VCT₅. This study confirms that dehydrogenase activity, which indicates the oxidation of organic matter, shows intensive activity during initial phases and declining activity towards the end, signaling vermicompost maturity. These findings align with previous vermicomposting studies (36,37).

V. CONCLUSION

This research aimed to bioconvert sugarcane bagasse, a sugar industry waste, with cow dung and the green manure plant *Gliricidia sepium* using the epigeic earthworm *Eudrilus eugeniae* to produce enriched vermicompost. Various combinations of vermibed materials were pre-decomposed with mushroom spawn (*pleurotus sajor-caju*) for 15 days before earthworm inoculation. The vermicomposting process lasted 50 days with the following treatments: VCT₁, VCT₂, VCT₃, VCT₄, and VCT₅. The microbial population (bacteria, fungi, actinomycetes) increased significantly for the first 30 days and then stabilized, with VCT₄ showing the highest levels. The organic substrates and earthworms facilitated rapid microbial colonization and activity, particularly in the mixture of sugarcane bagasse, cow dung, and *Gliricidia sepium*, resulting in nutrient-rich vermicompost. Enzyme activities (dehydrogenase, urease, acid phosphatase, alkaline phosphatase, protease, cellulase) were monitored, showing initial enhancement followed by a decrease towards the end of the vermicomposting. Enzyme activity varied between treatments, with VCT₄ consistently having the highest levels. The study concludes that the mixture of sugarcane bagasse, cow dung, and *Gliricidia sepium* is optimal for producing nutrient-rich vermicompost using *Eudrilus eugeniae*.

ACKNOWLEDGEMENT

We would like to express our sincere gratitude to the Department of Zoology, Annamalai University, for their invaluable support and provided facilities throughout this research project. This research would not have been possible without the collaborative effort and contributions from everyone involved. We are grateful for the opportunity to work with such a dedicated and supportive team.

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