RESEARCH PAPER

Impact of Biochar Amendment on Microbial Biodiversity and Humification Index in Vermicomposting of Sugarcane Bagasse

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ABSTRACT
India, as an agro-based country, produces large quantities of sugarcane bagasse (SCB), the fibrous residue of the sugar industry, which presents significant waste management challenges. Vermicomposting has proven to be an environmentally friendly method to convert such agro-industrial wastes into valuable compost. This study assessed the effect of biochar applied at different concentrations (0-6%) on the co-vermicomposting of sugarcane bagasse and cow dung using Eudrilus eugeniae. Microbial population dynamics and the humification index were monitored as indicators of compost quality and maturity. The results showed that biochar amendments notably affected microbial density and enzymatic activity, with the 4% biochar treatment yielding the greatest enhancement. Furthermore, humification, measured by HI, was accelerated in biochar-amended composts, with a decline of up to 90%, indicating improved compost maturity and stability. These findings illustrate that adding biochar at suitable levels can enhance vermicomposting efficiency, nutrient bioavailability, and microbial performance. Overall, biochar-enriched vermicomposting offers a sustainable approach to valorise sugarcane bagasse and cow dung, providing an effective solution for organic waste management while producing high-quality compost suitable for agricultural use.
Keywords: Bagasse, Biochar, Humification index, Amendment, Eudrilus eugeniae
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INTRODUCTION

India is an agro based country, with most of its rural inhabitants relying on agriculture as their source of livelihood. Among its key agro-industries, the sugar industry is a very important sector in the national economy. With about 705 registered sugar mills, India's annual production capacity is 180 lakh metric tonnes and is the world's second-largest producer of sugar after Brazil and the world's largest consumer [1,2]. Sugarcane bagasse is the fibrous residue left over after sugarcane stalks are squeezed for juice, and it has become an alternative resource for addressing waste disposal and sustainable raw material concerns [3]. Processing ten tonnes of sugarcane on average produces about three tonnes of bagasse which is mainly made up of 45%–55% cellulose, 20%–25% hemicellulose, 18%–24% lignin, with 1%–4% ash, and minimal amounts of waxes less than 1% [4]. Substantial amounts of these sugarcane wastes are routinely set on fire within the fields as a result of poor waste management techniques, with far-reaching environmental pollution and health concerns. In an effort to address these effects, the application of these wastes in low-cost, eco-friendly approaches such as vermicomposting has been suggested as a sustainable alternative [5,6]. Vermicomposting is a green bioconversion technique that encompasses synergistic earthworm and microorganism action to break down organic waste [7]. Apart from the improvement of mineral equilibrium and nutrient supply in soil, it also acts as a sophisticated biofertilizer that inhibits pathogenic organisms, restores soil vitality, and facilitates waste management, especially in cities. Microbial degradation of biodegradable waste is provided by extracellular enzymes, whereas in earthworms, the process takes place inside the alimentary canal, assisted by gut-associated microorganisms like fungi, actinomycetes, and protozoa[5]. The process leads to a nutrient-dense end product with higher microbial diversity and nutrient bioavailability than regular composting [8]. In particular, sugarcane bagasse vermicomposting is a natural, low-cost, low-labour, and effective method, where earthworms consume organic fibrous material and produce humus-like, black, and odourless vermicastings commonly known as black gold [9,10]. Earthworms are viewed as efficient and cost-effective vermicomposting agents because they have a high growth rate, high reproduction capacity, and can thrive in various environments [11]. Among the numerous influencing vermicomposting efficiency factors, choosing an efficient earthworm species is very important since the different

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species have different decomposition abilities. Growing research interest has been put on the use of earthworms, *Eudrilus eugeniae* not merely to biodegrade organic waste but also as a source of sustainable protein [12]. In this current research, *Eudrilus eugeniae* was utilized as the composting organism because of its established efficiency in degrading organic matter and tolerability to tropical conditions.

Among the various supplementary materials used to stabilize biowastes, biochar has gained considerable attention in recent years due to its multifunctional properties [13]. Biochar is conceptually and functionally different from other carbon-rich substances such as *charcoal*, which is commonly used for fuel, filtration, industrial reduction, or as a colouring agent, and black carbon, which broadly refers to all carbon-rich residues produced through combustion or thermal degradation processes [14]. It is a carbon-dense product formed via thermochemical biomass conversion technologies like pyrolysis [15]. In the recent two decades, biochar has evinced increased interest in agriculture owing to its ability to enhance the health of soil, sequester carbon, increase biosorption, purify wastewater, and facilitate sustainable innovations [16]. Several studies have indicated that the addition of biochar to organic waste treatment systems greatly enhances organic matter decomposition, physicochemical attributes, and microbial productivity [17]. Today, biochar is among the most efficient approaches to sequestering carbon long-term in farm soils [18]. Moreover, biochar enhances soil quality through enhanced water-holding capacity, electrical conductivity, and cation exchange capacity, thus ensuring plant nutrient retention and availability [19]. In this study, biochar was applied as an amendment to the vermicomposting system with sugarcane bagasse and cow dung with the objectives of improving compost quality, enhancing decomposition, and overall nutrient.

Despite the increasing use of sugarcane bagasse in composting, systematic studies on the impact of concentration-dependent effects of biochar amendments on microbial activity and compost maturity remain limited. The current study elucidates how varying biochar application regulates microbial diversity and humification index during the co-vermicomposting of sugarcane bagasse and cow dung. All these parameters are important markers of organic matter conversion, microbial efficacy, and compost maturity. By examining microbial population identification and determination of humification indices, the study gives a novel insight into the biochemical and microbial processes involved in enhanced compost quality and stabilization through the addition of biochar.

2. Experimental Procedures

2.1 Material Collection

The raw materials used in this study were sourced from different regions of Tamil Nadu. Bagasse was collected from the Kallakurichi Co-operative Sugar Mill in Moongilthuraipattu, Thiruvannamalai district. The earthworm species *Eudrilus eugeniae*, commonly known as the African nightcrawler, is a tropical, epigeic, detrivorous earthworm native to West Africa. It is widely recognized for its superior efficiency in vermicomposting under warm climatic conditions [20]. For this study, *E. eugeniae* was procured from VST Natural Farm in Kundumaranapalli, Hosur, Tamil Nadu. Cow dung was obtained from a local cattle farm and used as a bulking and nutritional agent. Previous research has indicated that cattle manure offers a more nutrient-rich and favourable environment for earthworms compared to goat manure [21]. The biochar used in the experimental treatments was sourced from Greenfield Eco Solutions.

2.2 Pre-processing of Bagasse

Precomposting is a significant phase of vermicomposting, as the majority of organic wastes need an initial phase of decomposition to be ready for earthworm processing. It typically involves the use of a bulking agent most commonly animal manure to enhance microbial activity and aeration [22]. Past research has proved that precomposting increases the decomposition of organic matter and significantly reduces the length of the entire composting process [23]. The bagasse was cut into small pieces measuring approximately 3-4 cm and subsequently spread on a clean surface that received indirect sunlight for a duration of five days. It was then pre-composted in compost bags for 30 days, during which moisture content was maintained by periodic watering to enhance nutrient availability prior to vermicomposting. Cow dung was allowed to air dry in the shade for a duration of 30 days to eliminate any harmful gases and heat that could adversely affect the earthworms. This precomposting stage helped to partially break down organic matter, such that the substrate was rendered more digestible and palatable for the earthworms during vermicomposting.

2.3 Experimental Setup for Vermicomposting

The experiment was designed to evaluate effect of twelve substrate combinations (C1 to C12) consisting of varied proportions of bagasse (BG), cow dung (CD), and biochar (BC). These mixtures were prepared and transferred into 36 grow bags with a holding capacity of 5 kg with each combination maintained in triplicates. The substrate mixtures involved sugarcane waste materials bagasse, supplemented with cow dung and biochar as conditioning and bulking agents. For every grow bag, 20 mature earthworms of *Eudrilus eugeniae* species were added to start the process of vermicomposting. The bags were kept under controlled environments with about 25 ± 3°C temperature. For effective decomposition, the moisture content was controlled between 60% and 70%, and water was supplemented as and when required during the process. The grow bags were left undisturbed throughout the composting process for efficient digestion of organic matter by earthworms. The mature vermicompost was air-dried, sieved to eliminate undecomposed materials, and stored for subsequent physicochemical and biological assessments.

Table 1. Composition of Vermicomposting Combinations (2Kg per vermibed substrates)

Composition	Bagasse: Cow dung	Biochar
C1	1:1	0%

C2	1:1	2%
C3	1:1	4%
C4	1:1	6%
C5	2:1	0%
C6	2:1	2%
C7	2:1	4%
C8	2:1	6%
C9	3:1	0%
C10	3:1	2%
C11	3:1	4%
C12	3:1	6%

2.4 Assessment of Microbial Populations

Microbial communities, including bacteria, fungi, and actinomycetes present in the vermibed substrate, were counted at the initial and final stages of the experiment with the standard plate count technique (Table 2) [24,25]. In analysing gut microbials, *Eudrilus eugeniae* content was poured into flasks with 99 ml sterile distilled water and then diluted in series using 9 ml sterile water blanks [26]. Aliquots of 0.1 ml, from suitable dilutions, were inoculated on sterile nutrient agar and incubated at 37°C for 48–72 hours. Plates containing 30–300 colonies were employed to determine bacterial counts per gram in CFU [27]. For substrate analysis, 1 g of each sample was suspended in 9 ml of distilled water, mixed on a vortex mixer for 30 minutes, and serially diluted (10^{-1} to 10^{-7}). 1 ml of each dilution was plated on selective media for bacteria, fungi, and actinomycetes. Every sample was tested in triplicate for accuracy.

Table 2. Microbial Enumeration: Media, Dilutions, and Incubation Periods

Parameter	Bacteria	Fungi	Actinomycetes
Media Used	Nutrient Agar	Martin's Rose Bengal Agar	Ken knight's Media
Dilution(s)	10^{-6}	10^{3}, 10^{-3}	10^{-4}
Incubation Time	1 day	3 days	7 days

2.5 Humification Index

The humification index (Q4/6) was calculated according to the method described by Zbytniewski and Buszewski [28]. One gram of each initial and final vermicompost sample was extracted with 50 mL of 0.5 M NaOH by shaking for 2 hours followed by overnight incubation. The extracts were then centrifuged at 3000 rpm for 25 minutes. Absorbance at 472 nm (A472) and 664 nm (A664) was measured using a UV-Vis spectrophotometer. The humification index was computed as Q4/6 = A472/A664. A smaller Q4/6 value implies higher degree of humification associated with the formation of aromatic, humic-like substances.

3. RESULT AND DISCUSSION

3.1 Microbial Community Analysis

Earthworm activity plays a crucial role in promoting microbial enrichment during vermicomposting by facilitating the efficient utilization of energy sources and restructuring microbial community structure in organic wastes. Flow of organic matter through the earthworm gut provides conducive physicochemical conditions, including ideal moisture, pH, and aeration, which foster the quick growth of desirable microorganisms. In comparison to aerobic microbial decomposition-based conventional composting, vermicomposting produces a more diverse and richer microbial community [29]. Microorganisms, including bacteria, actinomycetes, and fungi, are the principal agents that initiate biochemical changes in vermicomposting [30]. These microorganisms are capable of producing both extracellular and intracellular enzymes, which play a crucial role in the degradation of harmful organic compounds [31]. Therefore, vermicomposting is an environmentally friendly method of microbial enrichment for promoting sustainable soil fertility management by producing biologically active, microbe-dense organic amendments. Accordingly, the objective of the present study was to identify and assess the presence and dynamics of bacteria, fungi, and actinomycetes during the vermicomposting process.

The bacterial population (cfu × 10^{6} g^{-1}) of vermicompost from bagasse and cow dung with biochar amendment (C1–C12) showed an overall increasing trend during the vermicomposting process, reaching maximum values on day 45, after which it decreased gradually towards day 75. The initial bacterial populations varied from 43.22× 10^{6} cfu g^{-1}(C11) to 54.12 × 10^{6} cfu g^{-1} (C4).The highest bacterial population levels were achieved in combination C3, from an initial value of 49.56 × 10^{6} cfu g^{-1} on day 0 to a maximum of 156.32 × 10^{6} cfu g^{-1} on day 45, before declining to 104.25× 10^{6} cfu g^{-1} by day 75.The combination C3 also exhibited the greatest percentage increase at 110.35%, indicating that this combination strongly favoured bacterial growth and activity. Correspondingly, combinations C2 and C11 also demonstrated substantial percentage increases of 107.02% and 102.27%, respectively, suggesting good microbial growth in these combinations. Other combinations like C1 (82.17%), C6 (85.28%), and C9 (93.26%) also had quite good bacterial growth. Comparatively, lower percentage increases were in combinations C8 (65.19%) and C7 (78.20%) suggesting less favourable conditions for bacterial growth.

The fungal population (cfu × 10^{4} g^{-1}) in vermicompost prepared from bagasse and cow dung mixed with biochar (C1–C12) generally decreased throughout the course of vermicomposting, with the maximum number of fungi usually found at day 45 followed by a steep reduction on day 75 .Fungal population at the beginning of the experiment varied from 72.31× 10^{4} cfu g^{-1} (C6) to 84.51× 10^{4} cfu g^{-1} (C3). C3 had the maximum fungal population at day 45 at 144.21 × 10^{4} cfu g^{-1}, followed by C2 at 130.83 × 10^{4} cfu g^{-1} and C1 at 127.22× 10^{4} cfu g^{-1}.By day 75, fungal populations had reduced considerably in all combinations, with terminal counts varying between 54.06× 10^{4} cfu g^{-1} (C12) and 72.33 × 10^{4} cfu g^{-1} (C4). This overall reduction is reflected in the negative percentage changes observed in all combinations, with the greatest declines observed in C11(-28.98%) and C12 (-26.60%).

The population of actinomycetes (cfu × 10^3 g^{-1}) in cow dung and bagasse-derived vermicompost amended with biochar (C1–C12) increased during the initial stages of vermicomposting period, reaching a peak on day 45. After this peak, actinomycetes counts gradually declined on days 60 and 75.The initial population ranged from 61.22 × 10^3 cfu g^{-1} (C5) to 74.34 × 10^3 cfu g^{-1} (C3).The combination C3 had the highest counts, rising from 74.34 × 10^3 cfu g^{-1} on day 0 to 180.01 × 10^3 cfu g^{-1} on day 45 with a final count of 147.45 × 10^3 cfu g^{-1} on day 75 with percentage increase of 98.35%. Combination C4 had the greatest overall percentage increase of 119.67% with populations rising from 71.23 × 10^3 cfu g^{-1} to 156.47 ± 0.31 × 10^3 cfu g^{-1} by day 75. Other combinations like C2 (98.11%) and C6 (94.58%) also showed significant percentage increases. Lower percentage occurred in C11 (52.45%) and C12 (54.44%), implying that some proportions of substrate or biochar can suppress actinomycetes growth.

During vermicomposting, bacterial, fungal and actinomycete populations increased in the early stages, peaking around day 45 and declining by day 75. Bacterial and actinomycetes populations remained higher than their initial level at day 75, whereas fungal populations fell below their initial levels. The variations in the microbial populations observed during vermicomposting and composting are driven by a variety of factors that include the quantity and type of the substrate, the interactions between microorganisms, as well as the structural complexity of the organic material [32]. The microbial populations and diversity normally augment during the initial to middle stages of vermicomposting because of the presence of easily accessible nutrients and optimal environmental conditions. Gopal et al [33] stated that bacterial diversity was maximum on day 75 in vermicomposting of cow dung slurry and coconut leaves but decreased subsequently, presumably due to loss of moisture through air-drying, which is detrimental to microbial activity. Likewise, Devi and Prakash [34] found an initial rise in microbial populations until the 40th day in vermicomposting of substrates such as teak leaf litter, paper mill sludge, and pressmud mixed with cow dung with subsequent stabilization or minor drop. This increase in microbial populations is due to the presence of readily degradable organic substances and ideal moisture and aeration, which favour microbial activity and earthworm activity. Following this peak, once the organic material is more stabilized and the nutrient content is reduced, the habitat is less conducive to microbial growth. In addition, the buildup of microbial products, low substrate quality, and potential changes in moisture content can reduce the microbial population. Soobhany [35]observed that earthworms accelerate grape marc decomposition, boosting microbial activity early in vermicomposting and this activity declines as the substrate is exhausted and compost matures. Vermicomposting supplemented with biochar may alter the micro-environment within the earthworm's gut, leading to changes in the functional microbial profiles of the castings and enhancing the overall vermicomposting process. However, limited research has explored the effects of biochar on organic matter degradation and microbial community composition during the gut digestion process of earthworms [36]. The variation in bacterial, fungal, and actinomycetes populations during vermicomposting is depicted below in Figure 1.

Figure I: Microbial population dynamics during vermicomposting of bagasse and cow dung amended with biochar. (1a) Bacterial count (×10^6 CFU g^{-1}), (1b) Fungal count (×10^4 CFU g^{-1}), and (1c) Actinomycetes count (×10^5 CFU g^{-1}) across different treatment combinations

3.3. Humification index

Humification refers to the process by which new organic matter is converted into stable, humic materials. As a result, the level of humification can be considered a good indicator of compost stability [37]. Humification during vermicomposting is crucial in enhancing the maturity and quality of the finished compost product. Earthworms, in association with microorganisms, enhance the decomposition and stabilization of organic materials and improve humus formation and decrease the humification index (HI) [38,39]. In the present study, the HI of cow dung and bagasse mixtures treated with biochar (C1–C12) exhibited a distinct and continuous reduction during the process of vermicomposting, reflecting active and continuous humification. The initial HI values varied between 10.341 ± 0.89 (C3) and 11.543 ± 1.02 (C9) on initial day. On Day 45, the values had dropped considerably to 1.721 ± 0.09 (C3) to 2.116 ± 1.09 (C12), and dropped further by Day 75 to 0.996 ± 0.04 (C3) and 1.338 ± 0.06 (C12). The reduction percentage of HI was significant in all the combinations, which varied from −87.81% (C12) to −90.41% (C2). This significant reduction indicates the efficient breakdown of high-molecular-weight organic matter and its transformation into stable humic substances, reflecting advancing stabilization and maturity of the vermicompost.

Figure 3 illustrate the humification index (HI) values during vermicomposting of bagasse and cow dung mixtures amended with various percentages of biochar. The data clearly demonstrate a progressive and significant reduction in HI across all treatments (C1–C12), reflecting continuous humification and stabilization of organic matter over time. The results of the current study are in agreement with those documented by Gong et al [40] who noted that the use of 10% biochar blend in co-vermicomposting of cattle manure and maize straw greatly increased humification, encouraged compost maturity, and minimized nitrogen loss.

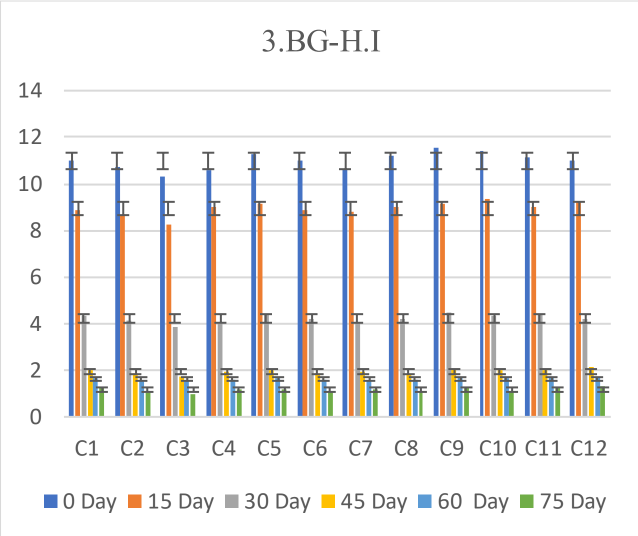

Figure 3. Humification Index of Bagasse and Cow Dung Mixtures Amended with Varying Percentages of Biochar

CONCLUSION

This study showed that the amendment of biochar increases enzyme activity, microbial diversity, and humification in the vermicomposting of sugarcane bagasse and cow dung. The most effective combination of biochar was 4% of C3 (1:1+4%), which presented higher activities of dehydrogenase, urease, acid and alkaline phosphatases, greater bacterial growth, and a larger decrease in the humification index; hence, it exhibited the best maturity of compost. 6% biochar (C4- 1:1+6%) favoured fungi and actinomycetes but did not improve the overall enzymatic activities or humification processes. Overall, moderate biochar addition optimizes microbial metabolism and organic matter stabilization, yielding high-quality, nutrient-rich vermicompost. The study was limited to short term, controlled conditions and did not evaluate the long-term performance of the vermicompost in field applications. However, this research verifies that biochar-amended vermicomposting is a sustainable and efficient method of managing sugarcane agro-industrial residues with the production of high-quality humified and nutrient-dense compost.

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