

THE ROLE OF MICROBIAL ENZYMES IN VERMICOMPOSTING OF ORGANIC WASTES

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<https://doi.org/10.5281/zenodo.10975242>

Abstract. *Using earthworms, vermicomposting (VC) is an environmentally friendly biotechnological technique that converts organic waste (OW) into vermicompost, a valuable, nutrient-rich fertilizer. Abiotic factors such as feed material, acidity, temperature, and moisture affect the process. Earthworms, and microbial communities work together to break down organic matter (OM). Consequently, a top-notch fertilizer is produced, which offers substantial benefits to soil health and the development of plants. Worm castings are a nutrient-rich fertilizer containing essential elements such as nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), and zinc (Zn). They also contain beneficial microorganisms in the soil and growth regulators that promote plant growth and development. The use of various worm species in composting allows for the treatment of different biological wastes, resulting in both environmental and economic benefits. Microbial enzymes such as proteases, cellulases, and polyphenol oxidases play a crucial role in breaking down organic material during composting, leading to improved efficiency and nutrient richness. Microbial communities, comprising bacteria, fungi, and protozoa, are responsible for organic material decomposition and nutrient cycling. Furthermore, the combined effects of various enzymes and microbial species enhance the composting process, resulting in better soil quality and increased nutrient availability. It is essential to comprehend the functions of microbial enzymes and important microbial species in process to optimize this disposal of waste method and its potential applications in sustainable agriculture and waste treatment.*

Keywords: *vermicompost, earthworm, enzyme, microbial communities, organic matter.*

1. Introduction

Vermicomposting (VC) is a biotechnological method that utilizes worms to change organic waste (OW) into nutrient-rich worm manure. Worms consume biodegradable waste and break it down into organic fertilizer, which is beneficial for organic farming and soil health. Worm castings contain essential nutrients such as nitrogen, phosphorus, potassium, calcium, magnesium, iron, manganese, and zinc, as well as beneficial soil microorganisms and plant growth regulators. Nevertheless, the method contributes to enhancing soil fertility, plant growth, and crop yield. Various worm species, such as *E. Fetida*, *E. Eugeniae*, *P. Excavatus*, *E. Andrei*, and *L. Rubellus*, can break down several types of biological waste. This method provides an economically viable and environmentally sustainable solution for managing solid waste, while also producing bio-fertilizers [1-4]. Although, microbial enzymes are essential for the composting of OW. They break down and degrade OM, facilitating the conversion of waste into nutrient-rich vermicast and vermishash [5, 6]. During the procedure, various enzymes, including proteases, ureases, hemicellulases, cellulases, esterases, and lignin-modifying enzymes, have been identified and studied for their functions [7]. However, these enzymes aid in breaking down various OW components, making them useful for biotechnological and industrial purposes [8]. The investigation focused on fungal and bacterial populations in sewage sludge composting. The aim

was to isolate strains with functional enzymatic capabilities. Ascomycota and Basidiomycota were the dominant fungal cultures, including species like *Aspergillus* and *Circinella*. High producers of laccases and peroxidases were *Thielavia* and *Bjerkandera*. The prevalent bacterial orders were Bacillales, Actinomycetales, Pseudomonadales, and Lactobacillales. Moreover, Efficient enzyme production was observed in species such as *Bacillus* and *Pseudomonas*, highlighting the potential of unique microorganisms [9]. An examination was carried out to investigate the impact of organic compost on the microbial features and enzyme activities of maize pot culture soil. The study demonstrated significant enhancements in various parameters, including dissolved organic carbon, available nitrogen, phosphorus, microbial carbon, nitrogen, catalase, invertase, and urease activities, as the levels of organic fertilizer, particularly at 5%, increased. Although there were no significant alterations in the metabolic quotient and the microbial C/N ratio, the vermicompost produced from biological waste significantly improved soil respiration rates. This suggests that it is effective in enhancing soil quality for grain production [10].

2. Methodology

In this review, we conducted an extensive analysis of nearly 150 academic articles obtained from Google Scholar and Semantic Scholar. We carefully scrutinized the existing literature to gather relevant information and selected a total of 77 articles from the initial pool based on specific criteria. Our focus was on the function of microbial enzymes in the composting of OW. The selected articles provided valuable insights into this area of study.

3. Vermicomposting

Composting is the process of using earthworms to convert OW into nutrient-rich vermicast. This sustainable and eco-friendly practice offers significant agricultural and environmental benefits [11-13]. Both worms and vermiculture are called vermes in Latin. It is more widely utilized today because it can manage a variety of OW by using different types of earthworms. These wastes include weeds, animal excrement, food waste, and agricultural waste [14]. It improves soil structure, stimulates plant growth, and reduces the impact of heavy metals, making it an essential element of sustainable agriculture [11]. Furthermore, it has been successfully used as a sophisticated biological solution for treating industrial waste, especially waste produced by the leather industry, demonstrating its potential in the field of waste management [15]. When different raw material combinations are used, they significantly affect the range of the worm manure, causing changes in electrical conductivity, pH, and nutrient levels [16]. Furthermore, composting has been found to effectively reduce nitrogen losses and greenhouse gas releases. This is mainly due to the significant impact of worm density, humidity, and carbon level on the composting process [17].

3.1 Mechanisms of vermicomposting

Basically, composting is divided into two phases: the active phase and the maturation phase. Throughout, the active phase of composting, biological waste undergoes degradation by earthworms and microbial populations. During the active phase of this stage, bacterial populations undergo significant changes and exhibit a wide functional variety, resulting in the production of high-quality fertilizer from decomposing organic material [18]. However, during the maturation phase of composting, OW is transformed into mature vermicompost, which is a high-quality organic fertilizer. Several techniques have been explored to examine vermicompost maturation. For example, a study showed that composting palm oil mill byproduct led to a decrease in the C: N ratio and improved nutrient recovery, indicating the maturity of worm manure [19].

3.2 Environmentally friendly worms

Around the globe, more than 3627 different types of land-dwelling worms exist. Because, they may help agriculture, these animals are frequently referred to as "farmer's friends". Earthworms comprise 60-80% of the aggregate biomass within soil and can exist in a variety of soil types. They can also be used as an indicator for the condition of the soil [20]. Worms and other soil creatures are essential for the provision of ecosystem services, such as the removal of toxic materials. Moreover, these organisms aid in the growth of plants by cycling nutrients, supplying necessary nutrients, controlling water, and absorbing carbon [21]. Nevertheless, because of their high rate of OW digestion, the high rate of reproduction, and ability to endure environmental challenges, earthworms are essential in the management of OW. These microorganisms produce various enzymes, including cellulase, amylase, invertase, protease, and phosphatase [22]. However, the study discovered that animal manure and sewage sludge composting process benefits from the presence of worm's *E. fetida*. Earthworms enhance stability and hasten the breakdown of organic materials [23].

3.3 The function of earthworms in the decomposition of organic material

Soil organisms, such as worms have an important role to play in the ecosystem services by eliminating harmful substances. They also aid in plant growth through nutrient provision, water regulation, carbon capture, and nutrient cycling. Additionally, they promote biodiversity, which is essential for ecosystem health. These services are crucial for ecosystem function, emphasizing the significance of preserving healthy soil communities [21]. Earthworms are practical for treating sewage sludge and domestic livestock effluents. They are also indicators of stable soil ecosystems. During composting, earthworms accelerate waste breakdown into nutrient-rich manure, acting as bioreactors. This transformation modifies waste's physiochemical properties. However, microbes are primarily involved in the biochemical reactions that lead to waste decomposition, but worms also have an important function in aerating, conditioning, and fragmenting the substrate, affecting microbial activity. Using worms as natural bioreactors for waste treatment shows promise in waste management [24]. Although, composting contains a diverse range of microorganisms, such as bacteria, fungi, and protozoa, which are crucial for the decomposition and nutrient cycling processes. These microorganisms assisting in the degradation of biological material leads to the release of nutrients and the suppression of harmful pathogens [25-29]. The worms significantly influence the decomposition of OM. Specifically, the impact of two different worm species, *E. fetida* and *E. eugeniae*, on the decomposition process has been extensively studied [30]. Furthermore, it has been demonstrated that epigeic earthworms residing in the boundary between litter and soil can affect the degradation of OM and its interaction with minerals, which may enhance the sequestration of soil organic carbon [31]. A further study found that the presence of different epigeic earthworm species, such as *M. hilgendorfi* and *E. fetida*, can affect the dynamics of soil carbon and nitrogen. This effect is evident in alterations in microbial communities and the accumulation of nitrate-N [32].



Fig. 1 vermiculture of E. fetida during the process of composting

Figure. 1 shows the decomposition of biological waste by composting with the earthworm *E. fetida* and its vermiculture.

2.3 The significance of microbial communities in vermicomposting

The process depends on the microbial communities shaped by the earthworm gut. These communities are crucial in breaking down biological waste and producing high-quality vermicompost. However, adding microbial associations to fresh OM can alter the function and diversity of microbial populations in VC technique [33]. Worms play a key role in stabilizing medicinal herbal residues while composting, which ultimately alters the microbial composition [34]. Earthworms are essential to composting because their gut acts as a bioreactor. This process depends on the symbiotic relationship between earthworms and microorganisms, including bacteria, fungi, and actinomycetes. These gut microbes assist in breaking down and digesting the OM consumed by earthworms [35]. These microbes also contribute to nutrient solubilization, nitrogen fixation, and suppression of plant pathogenic microbes [36]. The interactions between gut microorganisms and associated soil microorganisms play a significant function in the VC technique [37]. In addition, the use of microbial amendment during composting has been shown to have a beneficial effect on the bioremediation of the industrial and agricultural waste, ultimately improving the quality of the resulting vermicompost [38].

4. Microbial enzymes and their role in organic matter degradation

Microbial enzymes are essential for breaking down OM. These enzymes consist of oxidoreductases, hydrolases, monooxygenases, dioxygenases, methyltransferases, lyases, peroxidases, and lipases [39, 40]. Enzymes are vital biological catalysts that are important in a variety of fields [41]. Earthworms and microbes produce microbial enzymes that are essential for breaking down toxic compounds and facilitating the bioremediation process in polluted soil [42, 43]. Although, Microbial enzymes from earthworms and their associated microbes are crucial for decomposing OW. The enzymes are responsible for the decomposition of intricate organic substances into more basic forms, which are readily assimilated by plants and various organisms within the ecosystem. Earthworms act as natural decomposers, secreting enzymes that aid in breaking down organic material. Additionally, the microbes living with earthworms produce their own enzymes, further facilitating the decomposition process. This symbiotic relationship underscores the significance of microbial enzymes in the natural recycling of biological waste [36]. The presence of worms in soil ecosystems is essential for the degradation of pesticide residues, as they release detoxifying enzymes in their gut and have a beneficial effect on microbial populations, thus acting as soil engineers [44]. Microorganisms such as bacteria and fungi are capable of producing both extracellular and intracellular enzymes such as esterases, laccases, dehydrogenases and oxygenases. These enzymes play a crucial role in breaking down harmful chemicals. The use of microbial enzymes for the biotransformation of xenobiotic pollutants offers significant advantages over the use of whole cells for detoxification purposes. The mechanisms of microbial enzymes, particularly laccases and hydrolases, for bioremediation have been extensively studied. However, further research is needed to fully understand the potential of microbial enzymes in degrading different types of contaminants [45, 46].

4.1 Parameters that influence enzyme formation and activity

Temperature, moisture content and pH are three specific environmental factors that promote the development and activity of enzymes throughout the composting process. VC is best carried out at a temperature of 20 to 30°C and a moisture content of 80 to 85% [47]. Furthermore,

it has been demonstrated that adding vermicompost to soil increases the activity of oxidative-reducing and hydrolytic enzymes such as dehydrogenase, catalase, protease, and urease, which results in increased enzymatic potential [48]. Additionally, it has been discovered that using compound microbial agents throughout the composting process improves enzyme activities. Treatments with microbial agents showed greater levels of cellulose, laccase, manganese peroxidase, and lignin peroxidase when compared to controls. When combined, these elements provide an atmosphere that is favorable to effective enzymatic activity and production during the composting process [49].

4.2 Identification of key microbial species and their enzymatic activities

The process of composting is an efficient means of waste management that uses earthworms and associated microorganisms to break down organic materials. Bacteria, fungi, actinomycetes and other microorganisms are key players in this process, each fulfilling different roles. In particular, these microorganisms produce enzymes that accelerate the breakdown of organic material, thereby facilitating the decomposition process [25]. Fungi from maize grains, where *Fusarium verticillioides* was found to be the predominant species and *Cladosporium* and *Penicillium* were the best for lipase production. Bacteria from the digestive tract of a freshwater fish, where *Bacillus* was the predominant genus and showed high protease, lipase, amylase and cellulase activities [50, 51]. They also contribute to nutrient solubilization and nitrogen fixation, making the resulting vermicast nutrient-rich [52]. Although, the beneficial micro-organisms found in vermicompost play a key role in inhibiting harmful plant pathogens, ultimately improving the overall health of both soil and plants [6]. Proteobacteria, Bacteroidetes, Verrucae and Actinobacteria are among the various microbial groups that have been identified in the VC process [29]. Nitrogen-fixing bacteria such as *Rhizobium*, *Mesorhizobium*, *Bradyrhizobium* and *Azospirillum* have also been found in vermicompost [5]. Overall, the presence and activities of these microbes are essential for the successful decomposition and conversion of biological waste into nutrient-rich fertilizer through composting [36]. These microorganisms play a key role in the breakdown of organic material and are involved in the degradation of various components such as lignin, cellulose, hemicellulose and proteins [5]. The enzymes produced by these microorganisms, such as cellulases, hemicellulases, proteases and ureases, are important for the decomposition and conversion of OW into nutrient-rich compost. Understanding the activities and roles of these enzymes is crucial for optimizing the composting process and maximizing its potential for waste treatment [7]. A number of proteobacteria, including *Bacillus*, *Pseudomonas* and *Acinetobacter*, have been identified as key players in composting, contributing to the breakdown of OM and the production of beneficial enzymes and hormones [20]. These bacteria are involved in the breakdown of cellulose and the conversion of nutrients such as carbon and nitrogen, as evidenced by their production of cellulase, protease and other enzymes. The review paper do not directly discuss the involvement of Bacteroidetes in composting. However, it does highlights the importance of different bacterial strains in the composting process, particularly in the synthesis of cellulolytic enzymes [53]. Actinobacteria, particularly the genus *Micromonospora*, in the production of hydrolytic enzymes that are crucial to the composting process [54].

4.3 Synergistic effects of different enzymes and microbial species on vermicomposting

A number of investigations have extensively investigated the combined influence of different enzymes and microbial species on the composting process. The use of vermicompost has been shown to significantly improve soil microbial properties and enzyme functions, ultimately

resulting in improved soil quality [10]. Moreover, the purpose of the trial was to determine how various OW, such as cow dung, grass, water weeds and municipal solid waste, in combination with lime and microbial agents, affect the chemical and biochemical features of worm manure. Among the substrates tested, cow dung proved to be the most effective for composting. The addition of lime and microbial inoculants resulted in higher nutrient levels in the compost, as well as increased phosphatase and urease activities [55]. The presence of cellulolytic fungi increased cellulase activity in compost was associated with higher vinasse content. However, cellulolytic bacteria in vermicompost, with a significant reduction in paper cup waste due to bacterial consortia action [53, 56]. In addition, composting has revealed the presence of highly efficient amylase producers, namely *Bacillus cereus* and *Aspergillus niger*, among other specific microorganisms [57]. Proteases were isolated from protease-producing bacteria in the compost soil, indicating that these microorganisms are a potential source of protease in composting. This was further supported by the identification of *Bacillus* strains known to produce proteases in a composting facility [58, 59]. Lipase, an enzyme that catalyzes the hydrolysis of triglycerides, is a key player in composting. Studies have shown that the production of lipase can be optimized by factors such as temperature and initial moisture [60]. Polyphenol oxidase (PPO) is an enzyme that plays a crucial role in VC, particularly in the degradation of humus-PCP complexes. This process is further enhanced by the presence of earthworms, which help to accelerate microbial biomass and activity. In addition, the composting process can lead to the production of high quality organic biofertilizer and bioactive polyphenols, which are beneficial to plant growth and health [61, 62]. However, microbially derived peroxidases in particular are highly sought after due to their stability and versatility. Therefore, the function of peroxidase in composting is likely to be to break down OM and improve nutrient availability, contributing to the overall efficiency of the process [63]. Table.1 shows the "Diversity and function of enzymes in vermicomposting: Origins from earthworms, bacteria and fungi".

Table. 1 Displays the function of different enzymes in vermicomposting

Enzyme	Function	Source	References
Cellulase	Cellulase is an enzyme that catalyses the degradation of cellulose and is a pivotal component in VC.	Fungi, bacteria	[53, 56]
Amylase	Amylase is an enzyme that is essential in the process of VC as it aids in the decomposition of organic matter by hydrolyzing starch molecules.	Earthworm, Bacteria	[57, 64]
Proteases	Proteases are enzymes that play a crucial role in the mineralization of proteins and providing nitrogen sources for microorganisms during composting.	Bacteria	[58, 65]
Lipase	Lipase is a crucial enzyme involved in the hydrolysis of triglycerides, playing a significant role in the composting process.	Bacteria	[66]

Polyphenol oxidase	Polyphenol oxidase (PPO) is an important enzyme involved in the process of VC. It specifically degrades humic PCP complexes.	Earthworm	[62]
Peroxidases	Peroxidases are enzymes that are essential for enhancing the digestibility of crop residues, offering significant benefits in the composting process.	Bacteria, earthworm,	[67-69]
Laccase	Laccase plays a crucial role in the degradation of vermicompost by efficiently depolymerizing humic acids and facilitating humification.	Bacteria, fungi	[70, 71]
Xylanase	Xylanase enzymes are essential for breaking down xylan into fermentable sugars, which helps earthworms decompose organic matter during VC.	Fungi	[72, 73]

4.4 Importance of microbial enzyme dynamics in vermicomposting

The microbial enzymes that promote composting by degrading the organic material influence the quality of the compost. Studies show strong relationships among microbial composition, enzyme performance, and earthworm influence on microbial growth. The key to improving VC processes and increasing the efficacy of the final product is to understand these dynamics [74, 75]. However, feed quality is important for maintaining microbial and earthworm populations [76]. The potential of bacterial consortia to enhance microbial populations and enzyme activities during composting. Together, these studies highlight the importance of microbial enzyme dynamics in composting, with implications for waste management and sustainable agriculture [77].

Conclusion

Composting is the process of using earthworms to convert biological waste into nutrient-rich vermicompost. This method offers significant benefits for organic farming, soil health, and waste management. It improves soil fertility, plant growth, and crop yield while reducing environmental impact and promoting sustainable agricultural practices. During composting, microbial enzymes produced by earthworms and associated microorganisms play a crucial role in breaking down OM. The enzymes involved in composting include cellulases, amylases, proteases, lipases, polyphenol oxidases, and peroxidases. These enzymes aid in breaking down complex organic compounds into simpler forms that are more easily accessible to plants and other organisms. The combined effects of different enzymes and microbial species in VC systems can significantly improve soil microbial properties and enzyme functions, leading to enhanced soil quality. The efficiency of composting is influenced by factors such as organic waste composition, microbial inoculants, and environmental conditions.

Recommendations and suggestions

It is essential to study the synergistic influences of microbial consortia on enzyme synthesis and efficacy in vermicomposting. This strategy could lead to more effective decomposition of organic matter and release of essential nutrients.

Further research is needed to fully understand the potential of microbial enzymes in degrading different types of contaminants.

Earthworm populations in vermicomposting systems can be effectively maintained and increased by implementing various strategies. This is crucial as earthworms play a key role in the dynamics of microbial enzymes and the decomposition of organic matter.

The use of microbial amendments in vermicomposting systems has been extensively studied to introduce beneficial microbial communities. This practice aims to increase the diversity and activity of enzymes, resulting in enhanced degradation of organic waste.

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