

**Efficiency optimization of vermitechnology for generation of
sanitized end product: An in sight on PAH (polycyclic aromatic
hydrocarbons) detoxification and microbial activity**

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Summary

Lignocellulosic substances occupy a substantial proportion of solid waste generated worldwide. Among various lignocellulosic wastes, spent mushroom substrate (SMS) and vegetable waste are recognized as vital components of the global stock of biodegradable wastes. The SMS is mainly constituted of cereal straws with some fractions of wood saw dust and cotton waste. On the other hand, vegetable waste is one of major constituents of the household wastes generated every year in the developing nations. The lignocellulosic wastes are generally composed of several natural polymers in which lignin, cellulose, and hemicellulose are the predominant components. Although these substances are renewable, their biodegradability is challenged by their crystalline nature. The crystalline structures of these substances are endowed by homo or hetero polymers linked through various bonds in which β -1,4 and β -1,3 glycosidic bonds are the predominant ones. These wastes are mainly disposed in the landfills or burned in open areas that lead to severe environmental consequences. Therefore, the need for exploring sustainable and efficient recycling technologies is ever increasing.

Discussing sustainable waste recycling technologies, vermicomposting is one of the most efficient and eco-friendly technology for conversion of various types of solid wastes into valuable materials for agricultural use. However, the efficacy of vermicomposting technology for rapid decomposition natural lignocellulosic waste materials has not been extensively studied. The vermitechnology relies on the conjoint activity of earthworms and microorganisms. Presence of earthworms greatly modifies the microbial community structure in the waste materials by releasing their active gut-associated microflora. The microbial diversity in the vermicomposts considerably varies depending on the earthworm species, earthworm population, feedstock characteristics, and reactor conditions. Earthworms are known for their efficient pollutant removal potential. This might be closely linked to the microbial activity and earthworm species used for vermicomposting. As such, there are considerable research gaps on these aspects. The present endeavor, therefore, was formulated to study the impacts of earthworm species and their initial stocking density on various aspects of biodegradation such as mineralization, humification, microbial diversity, and metal bioavailability. Moreover, focused studies were undertaken to assess the polycyclic aromatic hydrocarbon (PAH) detoxification vis-à-vis removal kinetics under vermicomposting

and to develop user-friendly mechanized vermireactor for valorization of lignocellulosic biomass. The research was conducted in four stages, wherein in the first stage SMS was vermicomposted with three epigeic earthworm species (*Eisenia fetida*, *Eudrilus eugeniae*, and *Perionyx excavatus*) and the biodegradation dynamics were evaluated based on the breakdown of crystallinity, nutrient availability, shift in microbial community profiles, and seed germination assay. It was evident that the nutrient (N and P) availability increased with concurrent reduction in total organic-C in all the vermibeds. Interestingly, pH declined in *Eisenia* and *Eudrilus* systems while it enhanced in *Perionyx*-vermibeds. Remarkable values implied that *Eisenia* and *Perionyx* mediated systems were effective in feedstock degradation. Moreover, prolific abundance of vital functional groups (C=O, NH, and OH) along with remarkable diversity in phospholipid fatty acid assay (PLFA)-derived in fatty-acid distributions and microbial communities were observed in the three vermicomposting systems. The Seed germination assay, however, indicated the preeminence of *Perionyx*-vermicompost over *Eisenia* and *Eudrilus*.

The second stage of the study was formulated to assess the impact of the initial stocking densities of two earthworm species (*Eisenia fetida* and *Eudrilus eugeniae*) on valorization lignocellulosic waste in regard to earthworm fecundity, nutrient dynamics, feedstock crystallinity, humification profiles, microbial community structure, and metal bioavailability. The earthworm population significantly increased under low stocking densities (5 and 7 worm/kg); but denser stocking (15 worm/kg) noticeably suppressed the population growth. The XRD-based crystallinity evaluation showed that feedstock degradation efficiency of *Eisenia* was prudent at 7 worm/kg stocking, while the 10 worm/kg stockings of *Eudrilus* was most effective. Interestingly, the 5 and 7 worm/kg stockings effectively balanced NPK-mineralization and C-humification kinetics; which was strongly regulated by microbial activity, their community shift, and fatty acid profiles. Correlation statistics indicated that earthworm stocking density-driven microbial variations strongly influenced metal removal in vermibeds. These results implied that 5-7 worm/kg stockings of earthworms produced high-quality sanitized vermicompost.

The third stage of the research was conducted to appreciate the earthworm-mediated mineralization-decomposition influence the PAH-bioavailability in the end product. As such, the PAH accumulation capacity of *Eudrilus eugeniae* has not been

studied earlier. The apportionment dynamics of 13 PAH compounds in aerobic composting and vermicomposting (*Eisenia fetida* and *E. eugeniae*) systems were investigated using novel budget equations. The PAH removal efficiency of vermicomposting was 2-3 folds higher than composting with concurrent microbial augmentation. However, the 4-6 ring compounds reduced more sharply (30-50%) than the 3-ring PAHs, and *E. eugeniae* was an equally competitive PAH-accumulator compared to *E. fetida*. The budget equations revealed that although the bioaccumulation capabilities of earthworms were retarded due to PAH exposure, earthworms facilitated PAH-immobilization in decomposed feedstock. The correlation analyses strongly implied that earthworm-driven mineralization-humification balancing and microbial enrichment could be the critical mechanism of PAH remediation under vermicomposting.

In the fourth stage of the research, the main aim was to see if a mechanized and regulated vermireactor is developed then how such effort is improving the technology for efficient transformation of lignocellulosic waste into high quality organic manure. The evaluation was carried out with *Eisenia fetida* as vermicomposting agent. In this context, an economically efficient mechanized vermireactor comprising of shredding and watering device was designed for 5-10 kg capacity in such a manner that the reactor can be uninterruptedly operated with a continuous supply ready vermicompost after the initial incubation throughout the lifetime of the reactor. The performance of the mechanized shredder-fitted perforated vermireactor (MSVR) was assessed based on chemical changes in the feedstock, microbial activity, bacterial genetic diversity, and metal removal in the finished vermicompost in comparison with two types of vermireactors viz. clay and paper paste made perforated-walled truncated cone shaped vermireactor (CPVR) and simple earthen truncated cone shaped reactor (EVR). The experiment was found to highly encouraging as the N, P, and K availability dramatically increased in the MSVR with concurrent reduction of total organic C. The 16S rRNA gene sequencing based metagenomic analysis revealed that overall species richness and occurrence of rare species in the feedstock were significantly promoted in the MSVR as compared to CPVR and EVR. Eventually, the beneficial impacts of MSVR-mediated vermicompost were substantiated in regard to seed vigor and relative root and shoot growth of the spouted green gram seeds. Therefore, the MSVR showed great promise as a commercially viable technology up gradation of the vermicomposting process.