ABSTRACT

Vermicomposting is a bio-oxidative process involving synergistic activity of earthworms and microorganisms, which results in production of high quality compost from varied kinds of biogenic feedstocks in quicker time as compared to traditional composting process. As such, vermicomposting is a formidable and eco-friendly alternative waste recycling technology because the end-product results in lower levels ofmetal and organic pollutants as compared to orthodox composting. It has been estimated that the biodegradable substances constitute a major proportion (59% to 64%) of the overall solid waste produced in developing countries; among which vegetable and agricultural wastes (e.g., paddy straw, spent mushroom substrates, etc.) are the predominant constituents. Consequently, the importance and utility of vermicomposting technology have been well recognized and the applicability of the technology is increasing in recent time for waste valorization. The major obstacle for recycling of such wastes is their lignocellulosic composition. The crystalline framework of lignocellulosic materials poses strong resistance to microbial decomposition. Therefore, one the major challenges of bioremediation techniques (i.e., composting and vermicomposting) is to break-down such crystalline frameworks as quickly as possible through augmentation vis-à-vis activation of microbial communities and their functional properties. As such, the microbial community structure and their functional traits greatly vary depending on earthworm species and the kinds of the wastes managed by the vermireactors. Similarly, microbial community and functionality are strongly substrate-dependent in aerobic composting systems. On the other hand, pollutant remediating potential of vermicomposting systems greatly alters depending on several factors (earthworm species, nature of pollutants, initial concentrations, substrate compatibility, etc.). Therefore, the efforts to optimize the vermicomposting process with special referenceto end-product quality and microbial activity are necessary to establish the technological feasibility of the process. Under these contexts, I pursued this research to study the effectiveness of vermitechnology for recycling of different mixtures of lignocellulosic wastes (spent mushroom straw, vegetable waste, and rice straw, etc.). The underlying mechanism of biodegradation has also been studied with respect to different earthworm species, their initial population density, and the shift in microbial community profiles. Finally, a modest effort has been made develop an adjustable mechanized vermireactor for rapid conversion of biological wastes into nutrient-rich biologically active organic fertilizer.

The general introduction stating the background of the problem, significance,

research gaps, research questions, and identified research objectives has been presentedin the first chapter of the thesis. The second chapter is an exhaustive review of literatures, while the third chapter describes the methodological strategies that were adopted to achieve the research goals; illustrating the whole research plan in brief. The fourth chapter describes the outcomes of the experiments addressing the variations and alterations in microbial diversity and physicochemical profiles of lignocellulosic waste (spent mushroom straw) vermibeds deployed with three different earthworm species. This is followed by the fifth chapter comprising the study regarding the impact of earthworm stocking density on end-product quality, heavy metal compositions, and microbial community structure during vermicomposting of vegetable waste and rice straw based substrates. The sixth chapter reveals the differential response of two earthworm species regarding polycyclic aromatic hydrocarbons (PAHs) removal by computing the PAH removal budget and the seventh chapter describes the performance of an innovative mechanized vermireactor for valorization of biological wastes. The finalchapter (i.e., 8^{th} chapter) summarizes the whole study emphasizing on the key findingsof the research and future scope.

Microbial diversity and nutrient mobilization in lignocellulosic waste (spent mushroom straw) based vermicomposting systems

The experimental details and results addressing the first objective (i.e., to evaluate the variations in spent mushroom straw decomposition and microbial community structure in response to different earthworm species during vermicomposting) of the research have been showcased in the 4^{th} chapter. In this study, the qualitative value of vermicomposted spent mushroom straw (SMS) mediated by three earthworm species (i.e., *Eisenia fetida*, *Eudrilus eugeniae*, and *Perionyx excavatus*) was evaluated on the basis of nutrient availability, microbial activity, phospholipid fatty acid (PLFA) profiles, and seed germination assays. Degradation profiles of the lignocellulosic substrate in the vermireactors were assessed by monitoring the changes in crystallinity and distribution of functional groups using X-ray diffraction (XRD) and Fourier transform infrared spectroscopy, respectively. Total organic carbon decreased with an increase in nitrogen

and phosphorus availability in all vermibeds. Interestingly, pH declined in the *Eisenia* and *Eudrilus* systems but increased in the *Perionyx*-vermibeds. Significant reductions in XRD- derived crystallinity index with profuse microbial enrichment and an abundance of various functional groups were clearly observed in the vermicompost with *Perionyx* followed by that with *Eisenia*. Moreover, PLFA illustrated significant variations in fatty acid distributions and microbial communities of the three vermicomposting systems. The seed germination assay showed the superiority of *Perionyx*-vermicompost over those of *Eisenia* and *Eudrilus* with respect to germination index and relative root-shoot vigor. The results suggest that SMS degradability was affected by the growth of a healthy microbial community through vermicomposting.

Effects of earthworm stocking density on end product quality, microbial community structure, and heavy metal composition during vermicomposting

The second objective (i.e., to understand the impact of earthworm stocking density on humification, nutrient enrichment, and microbial activity during vermicomposting and composting of lignocellulosic waste) of the research has been dealt in the chapter 5 of thethesis. The experiment conducted under this objective was designed to evaluate the impact of different stocking density (5, 7, 10, and 15 worm/kg) of two earthworm species (*Eisenia fetida* and *Eudrilus eugeniae*) on microbial activity and community structure; thereby, determining the metal removal, crystallinity, nutrient bioavailability, and humification processes in lignocellulosic waste-based vermibeds. Earthworm populationsignificantly increased under low stocking; while denser stocking (15 worms per kg) wasstressful. The XRD-based crystallinity analysis revealed that the cellulose-rich feedstock degradation efficiency of *Eisenia* and *Eudrilus* was remarkably prudent at 7 and 10 worms per kg stocking density, respectively. Moreover, the 5 and 7 worms per kg stockings effectively maintained the balance between nutrient mineralization and C-humification, which were strongly regulated by microbial activity, their community shift, and fatty acid profiles. The active microbial biomass was significantly high under denser stockings (15 worms per kg) of *Eudrilus* and thinner stocking of *Eisenia* (5 worms per kg). Correlation statistics indicated that earthworm stocking density-driven microbial variations strongly influenced metal removal in vermibeds. These results implied that low initial stocking of earthworms could be

effective to produce high quality vermicomposts.

Assessment of PAH-removal kinetics and budget during vermicomposting

The studies performed to achieve the third objective (i.e., to study the polycyclic aromatic hydrocarbons (PAHs) detoxification routes in *Eisenia fetida* and *Eudrilus eugeniae* mediated vermicomposting system) of the research have been presented in the sixth chapter. 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. Remarkable increase in bacterial, fungal, and actinomycetes proliferation in PAH-spiked vermibeds with parallel removal of the PAHs indicated that earthworm-induced microbial enrichment plays a vital role in PAH detoxification during vermicomposting. The correlation analyses strongly implied that earthworm-driven mineralization-humification balancing and microbial enrichment could be the critical mechanism of PAH remediation under vermicomposting.

Vermireactor improvisation towards technical fortification

The chapter 7 illustrates the results of the experiment performed to accomplish the fourth objective (i.e., to study the impact of vermireactor improvisation on bio-waste sanitization, microbial diversity, and nutrient bioavailability during vermicomposting) of the research. Here, I designed and developed a mechanized vermireactor in collaboration with a faculty member of the Mechanical Engineering department at Tezpur University. The idea was to develop a reactor that encompasses all necessary physical operations (grinding, mixing, turning, and watering) for expediting the decomposition process and facilitating earthworms to function with fullest capacity.

Another important feature of the reactor was to maintain the vermicomposting process incontinuous manner by incorporating the sieving component within the reactor. The details of the design and related elaborations are furnished in the seventh chapter of the thesis. The performance of the mechanized reactor was compared with conventional earthen vermicompost bins (i.e., reactors). Kitchen vegetable waste (as a representative of lignocellulosic biomass) was mixed with cow dung in 1:4 ratio and used as feedstock for the all the reactors. Eventually, the reactors were incubated with adult specimens of two earthworm species (*E. fetida* and *E. eugeniae*) at a 50:50 proportion and the incubation was carried out for 60 days. The changes in chemical and microbial properties were estimated in periodic manner. Moreover, bacterial diversity in the end product was assessed using 16s rRNA (V3 V4) sequencing based metagenomic analysis. The study revealed that nutrient (N, P, and K) bioavailability significantly increased in the end-product processed in the mechanized reactor with concurrent reduction ofpH and total organic C; implying that the mineralization process was greatly facilitated in the reactor. The microbial activity and earthworm fecundity were also remarkably promoted in the mechanized reactor. Moreover, the metagenomic analysis clarified that the microbial diversity in mechanized reactor vermibed was considerably greater than that of the conventional vermireactors. Considering the problem of waste management in cities the mechanized vermireactor would be a useful proposition.

Concluding note

The major findings of the whole research endeavor are:

- The study related to studied the lignocellulosic biomass (i.e., SMS) degradation mechanism and efficiency of three earthworm species that the earthworms (especially, *P. excavatus* and *E. fetida*) could efficiently degrade SMS in short period of time by disintegrating the crystal framework of lignocellulosic materials and augmenting nutrient bioavailability through diversification of beneficial microbial communities and enhancing the availability of vital fatty acids.
- Earthworms (*Eudrilus eugeniae* and *Eisenia fetida*) exhibited high PAH-accumulation potential and overall removal efficiency of vermicomposting

system was ~100% for the 4-6 rings PAH compounds. While, the removal of the 3-ring PAHs was highly dependent on their initial concentrations. According to the mass-basis budget estimation of PAH partitioning, earthworm-induced PAH- immobilization in humified organic matter was more effective remediation route than bioaccumulation under vermicomposting.

- The efficiency of vermicomposting process could be escalated by reducing the initial stocking density of earthworms. The experimental findings suggest that comminuting efficiency of earthworms increased when the initial population was 5-7 worms per kg. The NPK-mineralization-C-humification balance, microbial community diversity, fatty acid profile, and metal remediation were greatly facilitated under low stocking density of earthworms.
- The developed continuous-mode mechanized vermireactor would likely be a potential candidate for valuable recycling of solid waste; particularly in urban areas and hence the prototype can be developed and improved for commercialization in future.