Chapter 7

Vermireactor improvisation towards technical fortification

Introduction

The recent global solid waste generation report predicts that the rate of waste generation would be more than double than the rate of population growth by 2050 [1]. The world generates about 2.01 billion tonnes of solid waste annually with a per capita per day generation of 0.74 kg; while the country-wise per person per day waste generation widely varies from 0.11-4.54 kg. As such, the World Bank report reveals that more than 33% of the solid waste generated globally is ill-managed, thereby leading to severe environmental consequences [1]. The extensive report also reveals the composition of waste and their management scenarios greatly vary depending on the country's economic progress and overall it is clear that the expenses for waste management is exorbitantly high in low to medium income countries. For example, food and green waste shares about 44% of the total solid waste generation of the world; but the share of this component is 32% of the total solid waste generated in high income countries, 56% in low income, and 53% in lower-middle income countries, respectively. The overview of the scenario in south Asia, where India is the major nation in terms of population and geographical area, shows that 57% of the waste is food and green waste. Moreover, 75% of the total waste collected in South Asian countries are open dumped. Under such contexts, it is well-understood that the wastes are needed to be managed efficiently in ecologically sustainable manners.

Composting and vermicomposting are two widely used biotechnological process for waste-to-resource conversion[2]. Composting is creating optimum condition for activation activity and proliferation of the inherent microbial resources in the biomass vis-à-vis feedstock [3]. On the other hand, the synergy and interaction of earthworm and microorganisms are utilized in vermitechnology to produce high quality end product within a short time period [4]. The presence earthworms results in efficient grinding of the feedstock owing to the comminuting potential of the earthworms gizzards and the excretory behavior, which not only improve the nutritional quality but also biochemically enrich the end product [5]. So far, huge scientific knowledge has been generated and technical modifications have been pursued with composting [6].

Fitzpatrick et al., (2005) [7]reported that the 'Beccari Composting System', developed Giovanni Beccari in Italy, has been recognized as one of the earliest mechanized composting system that was successfully applied in Europe and the US. In India, the first successful attempt of developing composting pits, known as 'Indore Composting System', for large scale manure preparation from agricultural waste was reported in 1930s[8].Eventually, the Rotary Drum Composting reactor was developed in 1940s in the US[6]. The Drum Composting reactor was later re-developed with additional features for Indian condition [9]. A parabolic composting reactor was developed as an integral component of bio-toilet system for conversion of night soil into nutrient rich organic manure [10]. Recently, Zahrim et al. (2021)[11] reported the performance of a passive aerated composting bioreactor fitted with biomass turning device for maneuvering food waste. On the contrary, there are only a few reports on developing mechanized vermicomposting reactor for facilitated transformation of biosolids. After an exhaustive literature search only two papers on mechanized vermireactor could be retrieved. Abbasi et al. (2015)[12] developed an efficient high-rate mechanized vermicomposting reactor and claimed that 100% biomass could be converted into ready-to-use manure within 21 days. A large scale continuous flow-through vermireactor was designed for organic waste transformation in Zimbabwe [13]. However, both the reported designs are suitable for medium to large scale application and thus cannot be used for household waste management because of their space consuming size and dimension.

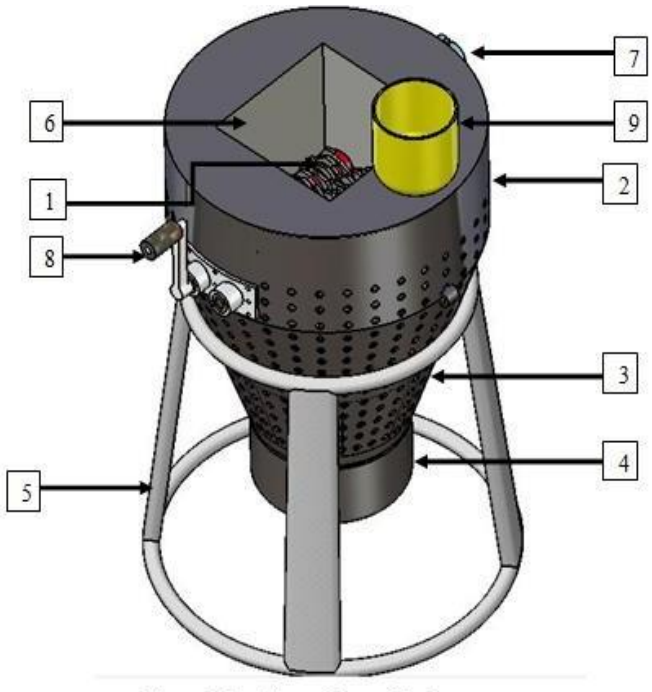
Therefore, an attempt was made to develop a continuous-mode mechanized vermireactor with turning and watering devices for rapid conversion of household wastes, keeping in mind that the household waste comprises the major proportion of municipal solid wastes generated worldwide [1]. The efficacy of the novel vermireactor was assessed in comparison with one conventional vermicomposting system and one modified aerated vermireactor. As such, the microbial activity and proliferation is the key to success of the vermicomposting process, which also indicates the performance efficacy of earthworm mediated systems [14]. Hence, microbial profiles were intensely studied in the finished products via 16S (V3 V4) rRNA based next generation sequencing approach. In addition, nutrient (CNPK) dynamics and earthworm fecundity in the vermireactors were periodically monitored for assessing the end product quality.

Materials and methods

Designing and proto-type development of mechanized and modified vermireactors

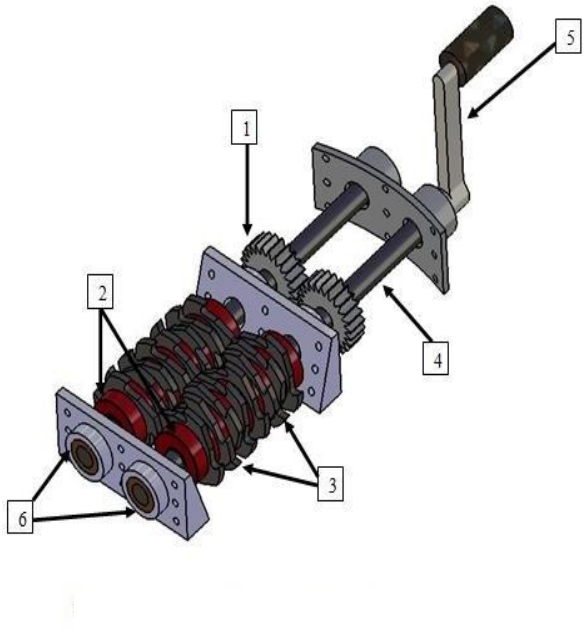
Mechanized shredder-fitted perforated vermireactor (MSVR)

An effort was made to develop a user friendly and economically efficient mechanized vermireactor that is comprised of shredding and watering device and can be used uninterruptedly with a continuous supply ready vermicompost after the initial incubation throughout the lifetime of the reactor. Considering the suitability and spatial arrangement for household use the reactor was designed for 5-10 kg capacity to ensure adequate aeration and ease of movement for the earthworms. Accordingly, the dimension of the various components was determined by using computer-aided solid model with an aim to maximize the space utilization. The design of the prototype has been described here in short with the basic diagram. The details of the design are not disclosed because the intellectual property of the innovation is yet to be protected, which would be applied in the nearest future. In short, different components (container, vermicompost collector, inner structure frame, shredder blades, bush, shafts, gears, fans, watering pipes, sensors, and the stand) were fabricated separately and then assembled together. Aluminum sheet and mild steel were mainly used for fabrication of different components of the reactor. Comprehensive diagrams of the solid model and dimension of different components have been presented in **Figure (7.3.2, 7.3.3, and 7.3.4) and Figure (7.3.6)**.As mentioned earlier, further details have not been provided in this thesis because of the intellectual property rights related legal issues.



1 – Shredder mechanism
2 – Upper part of the container
3 – Lower part of the container
4 – Collector
5 – Stand
6 – Hopper
7 – Fan
8 – Handle to operate shredder

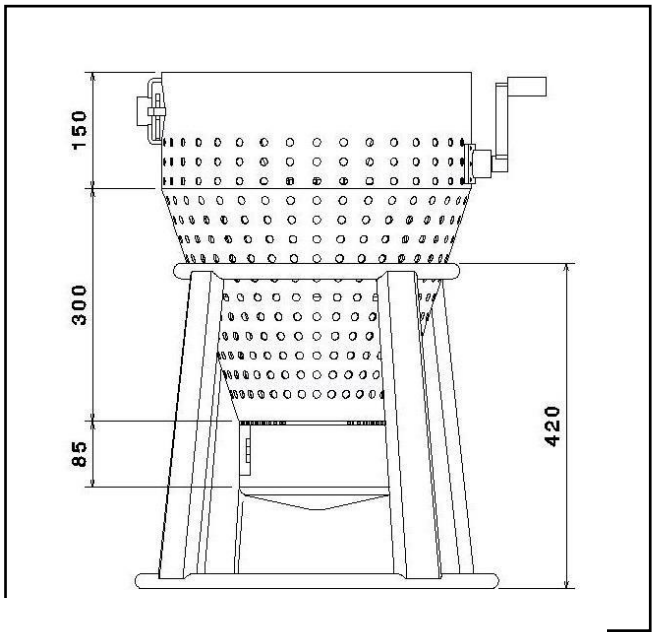
Fig:7.3.2 :The solid model of prototype



1 – Spur Gear
2 – Bushes
3 – Shredder Blades
4 – Shaft
5 – Handle
6 – Bearings

Fig:7.3.3 :The solid model of the shredder mechanism

Figure 7.3.4 – The solid model of the inner structure of the prototype



(All dimensions are in mm)

Figure 7.3.6 – Dimensions of the prototype.

Clay and paper paste made perforated-walled truncated cone shaped vermireactor (CPVR) and simple earthen truncated cone shaped reactor (EVR)

The earthen truncated cone shaped pots were taken as control. These were conventional flower pots without any perforation on the walls of 5-10 kg capacity. The clay and paper paste made perforated-walled truncatedrectangular vermireactors (CPVR) were specially designed with following dimensions – top width -1m; top length – 4.5m; bottom width – 0.5m; bottom length – 3.0 m; height – 0.9m (capacity – ~ 5-10 kg).The clay and wasted papers were collected from a nearby village pond and from the department of Environmental Science, respectively. Then, the clay and paper paste was made and eventually the reactors were made with help of a local potter by supplying him the design details. The picture and various dimensions of the CPVR have been presented in **Figures 7.3.7 and 7.3.1**

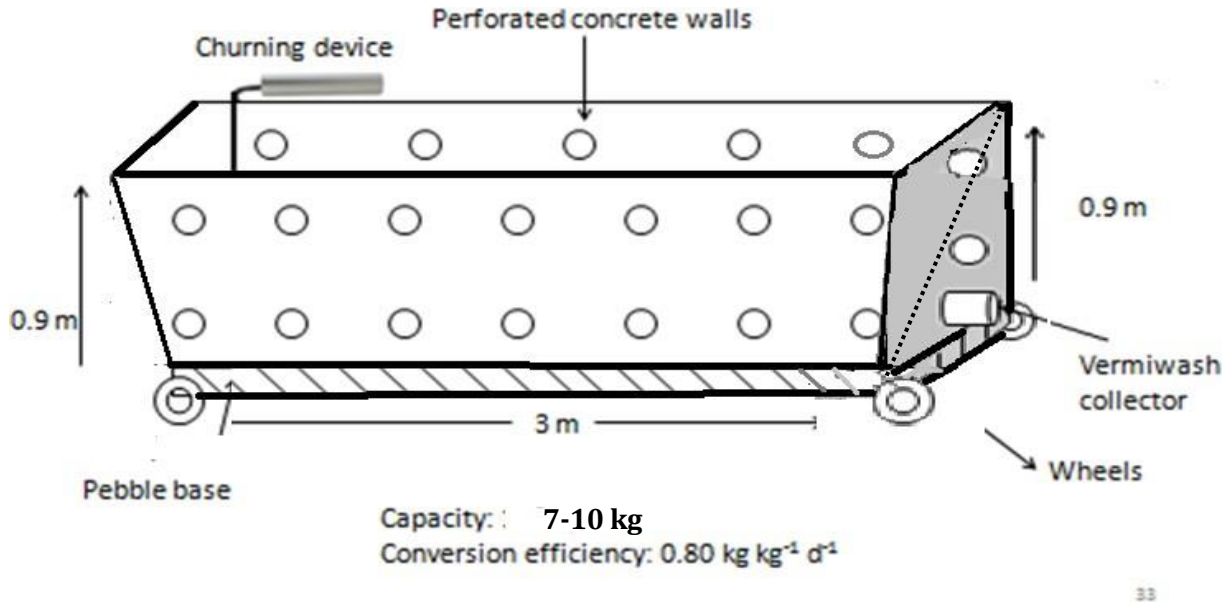


Fig 7.3.7: Clay and paper paste made perforated-walled truncated cone shaped vermireactor (CPVR)

Fig 7.3.1: Only earthen no perforated walled vermireactor (EVR) (Capacity 7Kg)

7.3 Feedstock preparation, earthworm selection, maintenance of vermicomposting systems, and sample collection

Vegetable waste (VW) and urine-free cow dung (CD) were collected from the local market and a nearby dairy farm, respectively. Eventually, the VW and CD were homogenized in 4:1 ratios in large quantity (~ 28-30 kg on fresh weight basis). Well-grown clitelleted specimens of *Eisenia fetida* were used for this experiment. Then, the feed mixtures were uniformly distributed @ 5 kg per reactor in the three types of vermireactors. The whole set was replicated thrice. The experiment was conducted for 60 days under shade. The moisture content was uniformly maintained at 50-60% for all the reactors. Accordingly, the watering pipes of the MSVR were regulated and for the CPVR and EVR water was sprinkled at 1-2 days intervals. Water was poured in the upper part of the container from where water was circulated via plastic pipes. The manually operated shredder of the MSVR helped in grinding the feedstock into finer pieces, which were thereby introduced in the container. The cylindrical-shaped upper part of the container was fixed with a fan and the earthworms were added in the truncated cone-shaped lower part of the container to carry out the decomposition process. As the wall of the container was perforated with 10 mm (diameter) holes hence optimum aerated condition could be easily maintained in the MSVR; which could be further facilitated by the fan fitted on the upper part of the container. The container was fitted with wired-net mesh at the bottom, which efficiently passed the granulated decomposed ready-to-use manure to the collector below (**fig 7.3.8**).

Fig 7.3.8: Mechanized with shredder and watering device incorporated vermireactors (MSVR)

However, aeration was maintained by churning the feedstock twice a day throughout the incubation period in CPVR and EVR. The Figure 3.9 provides the information about the experimental treatments and feedstock composition in nutshell. The feedstock samples from each vermireactor were periodically collected at 0, 30, and 60 d respectively. The collected samples were air dried, ground, and stored in air-tight polythene bags at 4^{o}C in a refrigerator to arrest the microbial decomposition process.

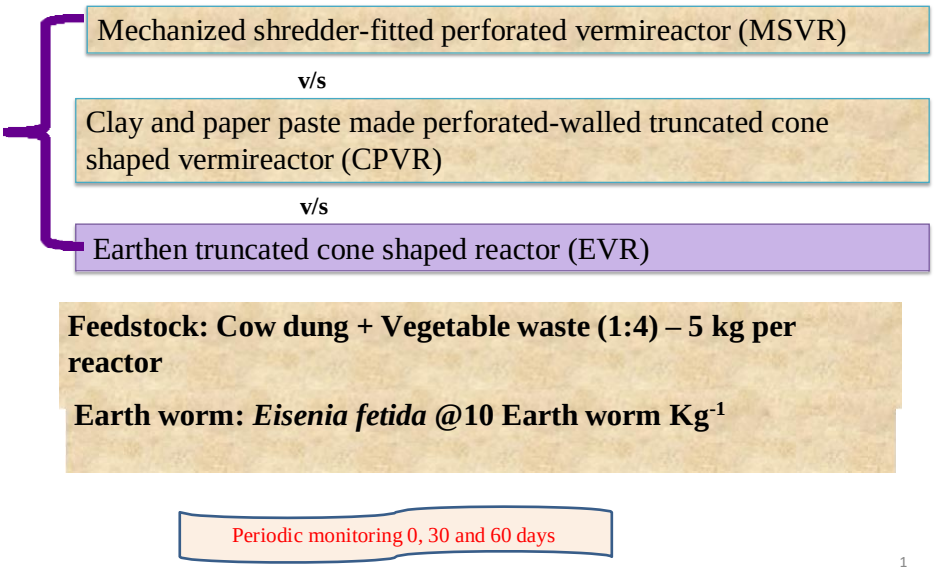


Fig 7.3.9 vermireactor experiment

7. 4 Earthworm fecundity

The growth and proliferation of earthworms were assessed based on the periodic counts of individuals and cocoons. The feed mixtures in different vermireactor were collected on aluminum trays at 30 d; then the worms and their cocoons were separated by sieving the feed mixture through iron mesh of 3-4 mm diameter and were re-constituted in the designated reactors along with the feedstock after counting. On 60 d, the matured vermicompost was collected in the collector chamber while the earthworms and cocoons remained in the main container of the MSVR were counted. However, the vermicomposted feedstock was manually removed from the CPVR and EVR and worms and cocoons were counted on 60 d.

7. 5 Chemical analyses

The changes in the chemical properties of the feedstock under vermicomposting was assessed on the basis of pH, total organic C (TOC), total N, available P, available K, DTPA extractable Zn, Mn, and Cd. The TOC, pH, available P, and available K were estimated after Page et al. (1982) as detailed in TOC section 4.3.2 under chapter 4, pH section 4.3.1under chapter 4, available P section 4.3.4 under chapter 4, available K

section 5.4.6 under chapter 5.The diethylene triamine pentaacetic acid (DTPA) extractable metals (Zn, Mn, and Cd) were estimated following the method standardized by Lindsay and Norvell (1978) in ICP-OES as detailed in section2.4.1

2.5.1**DTPA extract**: In this method, 1.967g of DTPA and 1.470 g CaCl2.2H2O were taken in a beaker. Then, 20– 25 ml of double distilled water (DDW) was added and thereafter 13.3 ml of tri ethanol amine (TEA) followed by 100 ml of DDW were also added. Then, the volume was made up to 1 l. This solution was adjusted to pH 7.3 and was used for extracting different trace elements remaining in bio-available forms.

PROCEDURE:

1. 20 gm of sample was taken in a 250 ml of conical flask.
2. 40 ml of DTPA extract was added to it.
3. It was then shaken in the mechanical shaker for 2 hrs. and then filtered.
4. Estimations of these elements were carried out with the help of AAS and ICP-AES.

Microbial analyses

The changes in microbial biomass C (MBC) was periodically analyzed in the feedstock samples by following the method described by Jenkinson (1988). The details of the method have been elaborated in section*4.5.2* under chapter 4. Moreover, the 60 d samples of the three different vermireactors were subjected to 16S rRNA gene based metagenomic analysis. Initially, the genomic DNA was isolated using the soil DNA isolation Kit (MO BIO Laboratories, Inc., USA) following manufacturer's protocol and the isolated DNA samples were stored at – 20 oC. Eventually, the library was prepared following the standard guidelines of the 16S Metagenomic Sequencing Library Preparation tool (Illumina Inc., USA) and the V3-V4 hyper-variable region of the bacterial 16S rRNA gene were amplified using standard primers(5' – CCTACGGGNGGCWGCAG - 3' and 5' – GACTACHVGGGTATCTAATCC- 3') [13]. Trimmed and filtered sequence reads were directly aligned to the 16S reference sequencesto perform abundance estimation and taxon classification. The alignment and taxonomicclassification was performed with Kraken2 and Bracken tools. The abundance plots for taxonomic classification were generated with R packagesphyloseq and ampvis2.

Seed vigor assay

The impact of the three vermireactors-driven vermicomposting systems on plant growth was evaluated based on a seed vigor (i.e., germination) assay as detailed in chapter 4 of the thesis. Likewise, fresh and disease free green gram (*Vignaradiata*) seeds were also used for the current experiment. As such, the assay facilitates to estimate useful attributes relative seed germination (RSG),relative shoot growth (RShG), and germination index (GI) [15]. Further details can be found in section 4.6 of chapter 4.

Statistical analysis

The data estimated for various chemical and microbial attributes (pH, TOC, total N, available P, available K, DTPA-extractable Zn, Mn, Cd, and MBC) were analyzed with two-way ANOVA followed by LSD post-hoc test as detailed in previous chapters.

Results and discussion

Impact of vermireactors on earthworm fecundity

The data on periodic changes in earthworm and cocoon counts in three types of vermireactors have been presented in Figure. In general, earthworm population significantly increased on temporal basis by 1.21-1.51 folds in all three types of vermireactors (p for days < 0.001; Fig.3.10).According to the two-way ANOVA the effects of vermireactor designs (i.e., treatments) on earthworm proliferation was also significant (p for treatment×day< 0.001). At the end, highest earthworm count was recorded in the MSVR followed by CPVR and EVR (p for treatment < 0.001, LSD = 0.74; **(Fig. 3.10 (a))**. Increase in earthworm population is a dependable indicator of worm compatibility to vermicomposting process [16]. Interestingly, the population growth of *E. fetida* increased at an increasing rate between 30 and 60 days in the MSVR; while such trend was absent in CPVR and EVR. Correspondingly, cocoon production remarkably increased in the MSVR over time followed by CPVR and EVR (p for treatment and day < 0.001, LSD = 0.52; **Fig.3.10(b)**). These results strongly imply that the ambient condition of the MSVR was highly favorable for the earthworm growth thereby facilitating the vermiconversion process in the MSVR.

(A) (B)

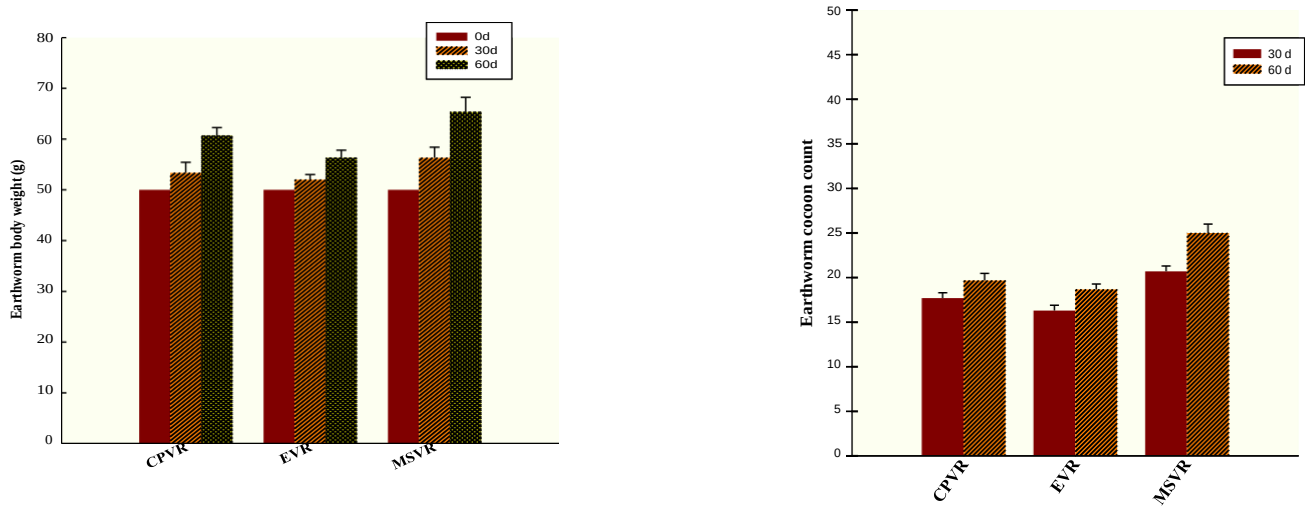


Fig.7.3.10. Temporal variation in (a) earthworm counts and (b) earthworm cocoon counts in the feedstocks under the composting and vermicomposting system .Error bars represent the standard deviation; LSD means Least Significant Difference**.**

Changes in chemical composition of the feedstock under vermicomposting

The data on changes in pH, TOC, and TKN in the feedstock of different vermireactors are presented in Figure. The pH of the feedstock was slightly alkaline in the initial stage which sharply increased till 30 d and then significantly decreased at 60 d in the CPVR feedstock (p for treatment and day < 0.01). In contrast, a consistent and significant decrease in pH was recorded in MSVR feedstock (1.26 fold) while the pH in EVR feedstock marginally decreased over time (1.11 fold) (**Figure 7.3.11 (a)**. At the end, the feedstock pH was in three reactors was in the order: MSVR > EVR > CPVR (LSD = 0.03). Reduction in feedstock strongly indicates efficient organic matter decompositiondue to vermicomposting [17]. Therefore, the constant pH decreasing trend in MSVR implied that the earthworm-mediated decomposition was more pronounced in the mechanized reactor than the CPVR and EVR. The TOC of the lignocellulosic feedstock was noticeably high (16.35-16.85%) at the initial stage (**Figure 7.3.11 (b))**. Such high initial TOC indicates the recalcitrant nature of the feedstock, which substantially resists the microbe-earthworm induced biodegradation process [14]. Eventually, the feedstock TOC remarkably reduced in all the vermireactors; while the

extent of TOC decrease was most remarkable in MSVR (83.49%) followed by CPVR (40.95%) and EVR (20.79%) after 60 days of vermicomposting (p for day and treatment < 0.01, LSD = 0.048; (**Figure 7.3.11 (b)**.This implies that earthworm and microbe mediated C mineralization was remarkably promoted in the MSVR feedstock followed by the CPVR feedstock (Khwairakpam and Bhargava, 2009). This result also indicates that the population increment of *E. fetida* in the MSVR probably expedited the decomposition of the recalcitrant lignocellulosic feedstock. This interpretation was in good agreement with a previous study [18].Correspondingly, the total N content in the feedstock remarkably enhanced under all the vermireactors by 5.7-15.7 folds over 60 days (**Figure 7.3.11 (c)**. However, the rate of N increment was significantly greater between 0 to 30 d than 30 to 60 d (p for time < 0.01). This indicates stabilization of the decomposition process during the later stage (Hussain et al., 2018). At the end, the total N content in the feedstock under three types of vermireactors was in the order: MSVR > CPVR > EVR (LSD = 0.22; (**Figure 7.3.11 (c)**). Earthworms increase N availability through release of active gut microorganisms and by breaking down the polysaccharides of lignocellulosic materials [19]. Therefore, the present results imply that earthworm-microorganism synergy was greatly promoted in the mechanized vermireactor (MSVR).

The P and K availability significantly enhanced in the feedstock as compared to the initial values after vermicomposting (**Figure 7.3.12 (a)and(b)**). The P content was inherently high in the initial feedstock which increased by about 3.73 folds in the MSVR followed by CPVR (2.44 folds) and EVR (2.02 folds) after 60 days (**Figure 7.3.12 (a)**). The initial availability of K was low in the feedstock (**Figure 7.3.12 (b)**) This may be due to the recalcitrant nature of the feedstock. Interestingly, K availability dramatically increased in the MSVR by 38.31 folds followed by CPVR (19.48 folds) and EVR (17.17 folds) after 60 days (LSD = 1.04). Lazcano et al., 2008 reported earthworms augment the growth and proliferation of P solubilizing microorganisms that results in acceleration of phosphatase-mediated mineralization of phosphoric esters. While physical breakdown of feed mixtures and secretion of exogenous/endogenous microbial enzymes are facilitated by the earthworms that result in remarkable K enrichment during vermicomposting [5]. Thus, it is inferred that the remarkable P and K enrichment in the MSVR system should be due to activated microbial growth and the associated release of extracellular enzymes.

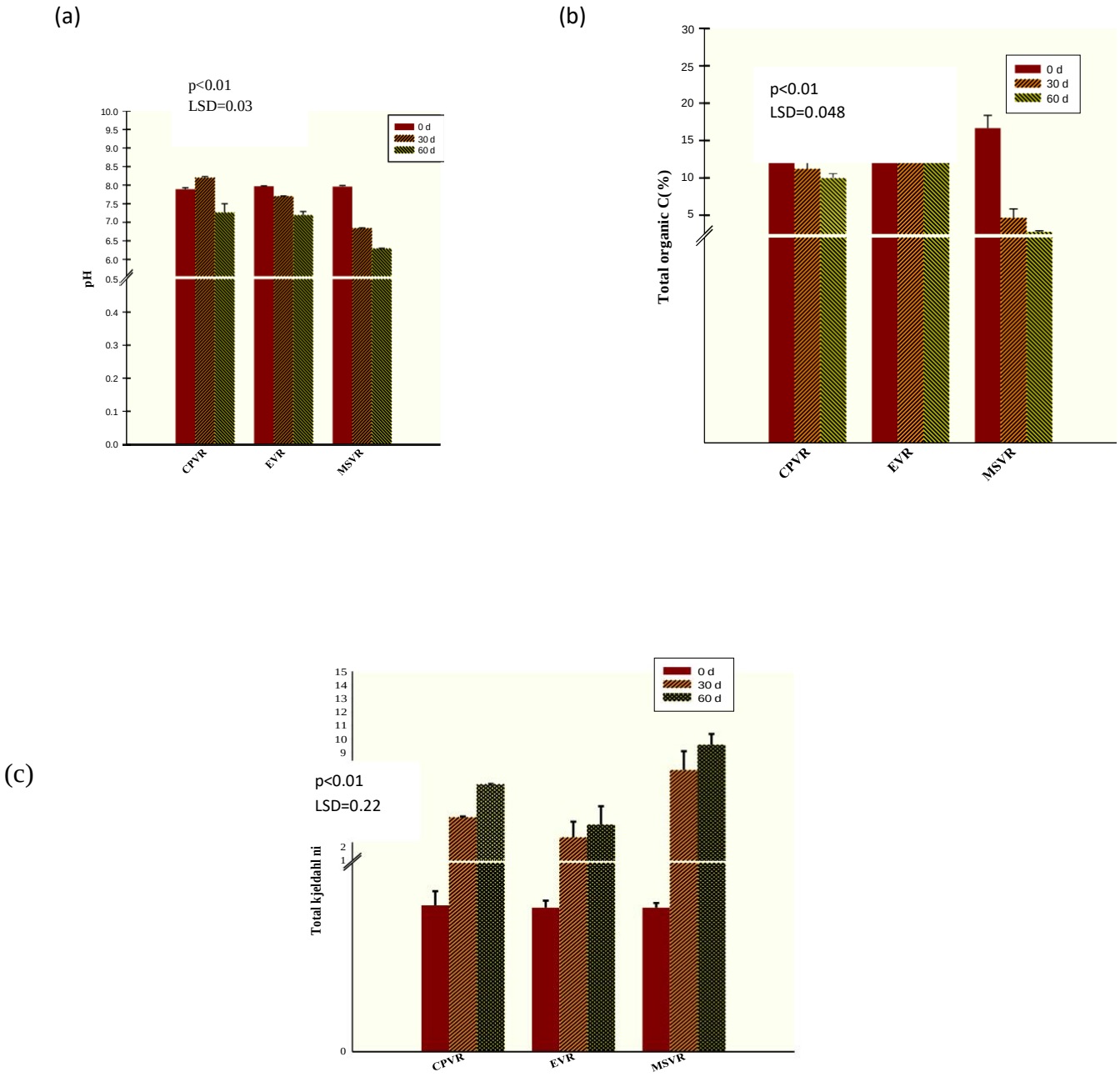


Fig.7.3.11. Temporal variation in pH, total organic C, and total Kjeldahl N in the feedstocks under the composting and vermicomposting system.Error bars represent the standard deviation; LSD means Least Significant Difference.

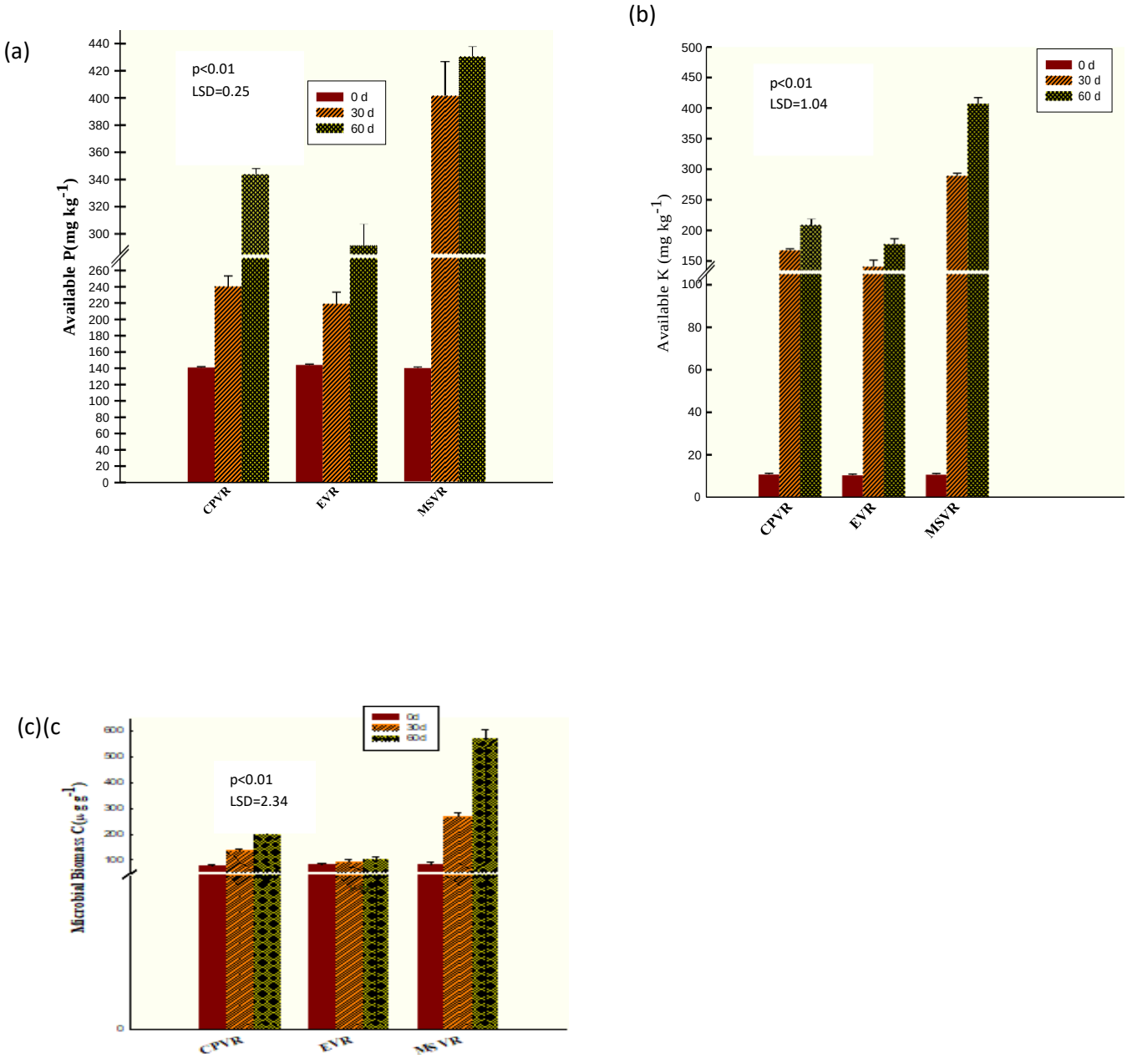


Fig.7.3.12. Temporal variation in (a)Avl P,(b) Avl K, and (c)MBC (Microbial biomass C) in the feedstocks under the composting and vermicomposting system Error bars represent the standard deviation; LSD means Least Significant Difference.

Impact of vermireactors on microbial enrichment

The data on changes in microbial biomass C (MBC) in the feedstock under different vermireactors are presented in **Figure.7.3.12 (c).**The initial low MBC in the feedstock implies that the obstinate nature of the lignocellulosic materials created obstacle for microbial proliferation, which is considered to be a big challenge for easy biodegradation

of such materials [17,18]. In general, the MBC significantly increased due to vermicomposting and according to the two-way ANOVA the time × treatment (i.e., reactor) was also significant ($P < 0.01$). Interestingly, the extent of MBC increment was most pronounced in the MSVR (570.14±34.21 µg g-1) followed by CPVR (196.57±11.79 µg g-1) and EVR (104.77±6.29 µg g-1) after 60 days of incubation (LSD = 2.34). Earthworms induce microbial growth in vermicomposting reactor via release of their intestinal microorganisms along with excreta [22]. Hence, the result clearly indicates that earthworm gut associated microflora and their subsequent release was greatly facilitated in the MSVR. Such spectacular result was an encouragement for studying the microbial profile of the three vermireactors in details using 16S rRNA based metagenomic technique **(Figures 7.3.13-7.3.17)**.

The major hypothesis of the 16S rRNA gene sequencing was that the V3-V4 primer set might identify various portions of microbial abundance in the unique environments of three different vermireactors. There was a striking difference in bacterial diversity at genus and phylum levels among the three vermireactors. At genus level, predominance of *Tumebacillus* was significantly high in the CPVR and EVR **(Figure 7.3.13)**. Steven et al., 2008 reported that the *Tumebacillus* are group of Gram-positive aerobic bacteria under phylum *Firmicutes*. Carper et al., 2020 reported that *Tumebacillus* species have been isolated from soil, permafrost, river water, and vulture gut. In contrast, the *Tumebacillus* genus was not detected in the MSVR, while the overall genus diversity was considerably greater in the MSVR as compared to CPVR and EVR **(Figure 7.3.13)**. High abundance of *Adhaeribacter*, *Sphingomonas*, *Brevenundimonas*, and *Lysobacter* was the interesting feature of genus diversity in the MSVR. The *Adhaeribacter* species have been found in soils and they are potential source of alkaline and acid phosphatases [23]. The genus *Sphingomonas* and *Brevundimonas*, consist of oxidase-positive Gram-negative bacteria, are widely found in various environments [21,22]; while the *Lysobacter* genus includes various rhizospheric bacterial species and are known to produce arrays of extracellular enzymes [26]. These results strongly suggest that the MSVR promoted the growth of numerous useful bacterial genera that may play vital role in accelerating the mineralization of obstinate feedstock through enzyme activation. Interestingly, the overall abundance of genes **(Figure 7.3.14)** in the feedstock considerably differed among the vermireactors. The abundance of *Arthobacter*, *Ferruginibacter*, *Edaphobacclum*, *Lysobacter*, *Bacillus*, *Brevundimonas*, and

Sphingomonas genera was more prudent in the MSVR than CPVR and EVR. Interestingly, some genera (e.g., *Luteimonas*, *Pontibacter*, *Pedobacter*, *Lysinibacillus*, *Fictibacillus*, and *Solibacillus*) were absent while some (*Caulobacte*r and *Aridibacter*) were exclusively found in the MSVR feedstock **(Figure 7.3.14)**. Moreover, the *Pseudomonas* genus was detected in MSVR and CPVR but not in EVR; while *Pseudoxanthomonas* and *Microbacterium* could be detected in MSVR and EVR but not in CPVR. This observation implies that the earthworm-induced modification of bacterial community profiles may vary depending on reactor condition.

At phylum level, it was interesting to note that the overall phylum diversity was substantially greater in the MSVR than CPVR and EVR **(Figure 7.3.15)**. *Firmicutes* was one of the predominant phyla along with *Proteobacteria* and *Actinobacteriota* in CPVR and EVR. Contrastingly, occurrence of the members of the *Firmicutes* was drastically reduced with concurrent rise in *Proteobacteria* followed by *Acidobacteriota*, *Chloroflexi*, and *Actinobacteriota* phylum in the MSVR feedstock. Moreover, the *Planctomycetes* (i.e., *Planctomycetota*) phylum was exclusively found in the MSVR feedstock. The members of the *Firmicutes* are typical fast-growing copiotrophs that are incapable to proliferate under low carbonaceous substrate due to their low substrate affinity and dearth of regulatory efficiency under starvation [27]. On the other hand, oligotrophic groups (*Proteobacteria*, *Acidobacteriota*, *Chloroflexi*, and *Actinobacteriota*) are known for their high substrate use efficiency and thus can transform unavailable (i.e., organically bound) nutrients into their bioavailable forms more efficiently than the copiotrophs [24,28]. Therefore, predominance of oligotrophic bacterial communities in the MSVR was a strong evidence of the better nutrient enrichment facilitation in the MSVR compared to CPVR and EVR. In particular, augmentation of *Planctomycetota*and *Acidobacteriota* in the MSVR feedstock suggests that the reactor condition might have promoted the growth of ecologically significant bacterial communities. The *Acidobacteriota* play vital role in modulation of the bio-geochemical cycles in the plant-soil ecosystems [26,8]. While the members of *Planctomycetota* phylum play significant role in the global C and N cycles and many species of the phylum readily oxidize the anaerobic ammoniacal nitrogen [30].

The bacterial compositional fluctuations among the vermireactors could be comprehensively appreciated by studying the taxonomic and phylogenetic diversity

(Figure 7.3.16 and 7.3.17). The **Figure 7.3.16** shows the alpha-diversity where both Simpson and Shannon diversity indices have been estimated in the samples. The Shannon measure is sensitive to rare species while the Simpson measure is sensitive to common species. Interestingly, these measures exhibit that proliferation of both rare and common species was more facilitated by the MSVR rector followed by CPVR and EVR. In general, the presence of epigeic earthworms like *Eisenia fetida*rapidly mobilize bacterial diversity in the vermibed with a steady and stable increase in richness and diversity of bacterial communities [28]. The rarefaction curve developed by estimating the species richness in the samples strongly substantiate that the MSVR reactor greatly enriched bacterial communities, which finally resulted in producing more enriched vermicompost than CPVR and EVR **(Figure 7.3.17)**.

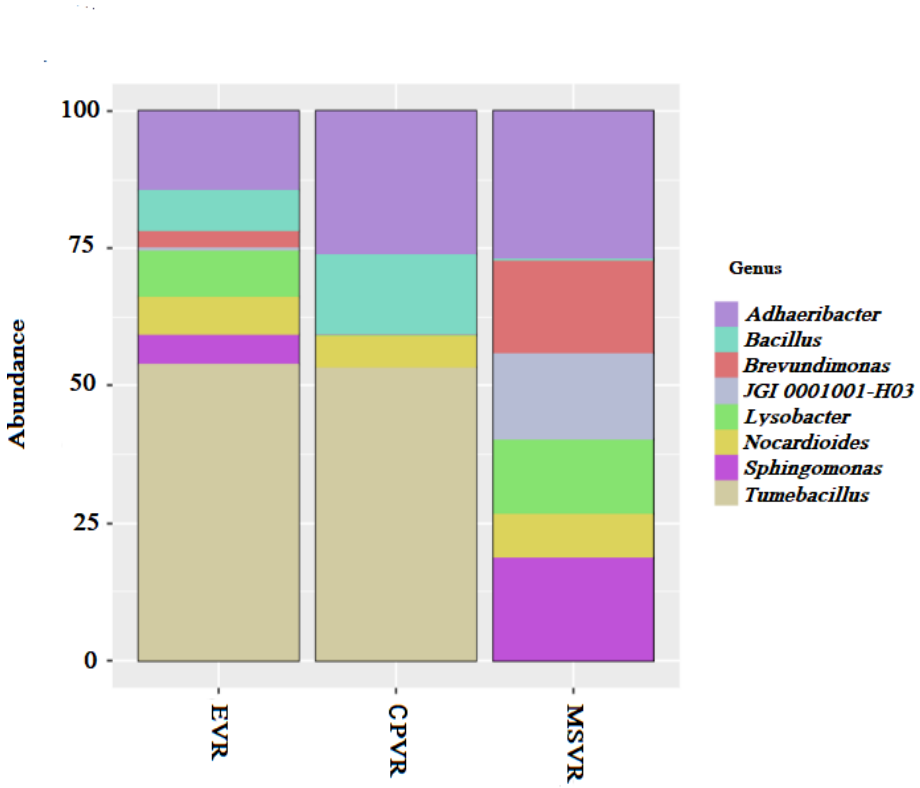


Fig 7.3.13: Relative abundance distribution of detected OTU by Taxon classification Genus

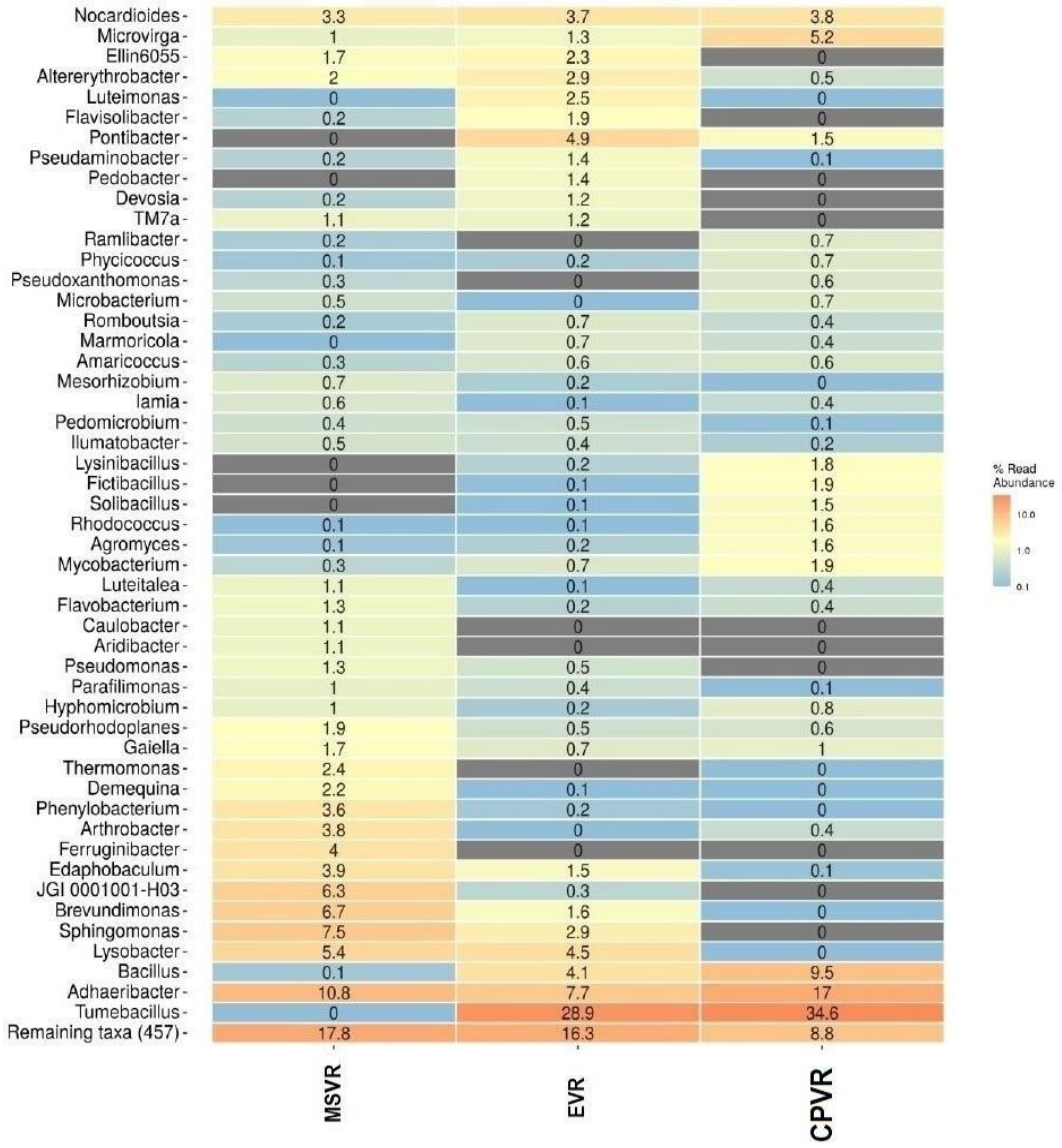


Fig 7.3.14: Abundance of genes in the vermireactors derived from sequenced data

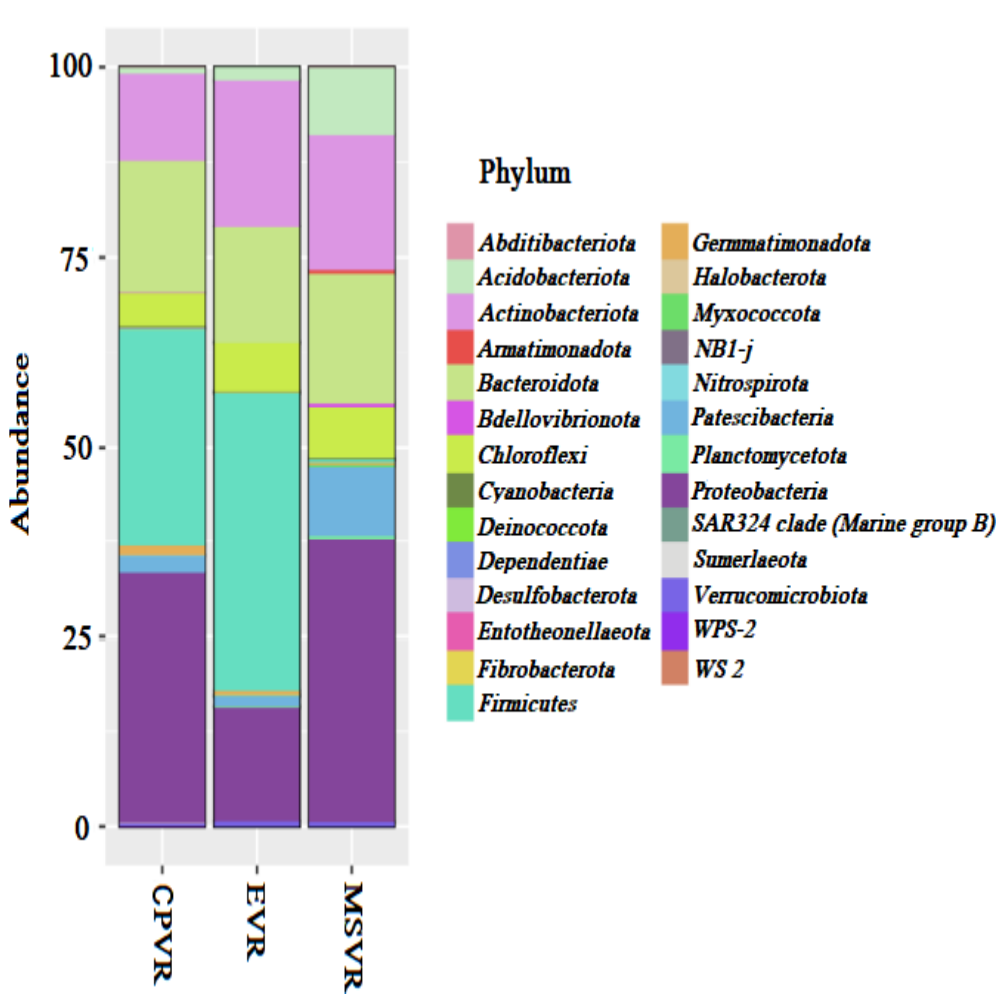


Fig 7.3.15: Relative abundance distribution of detected OTU by Taxon classification Phylum

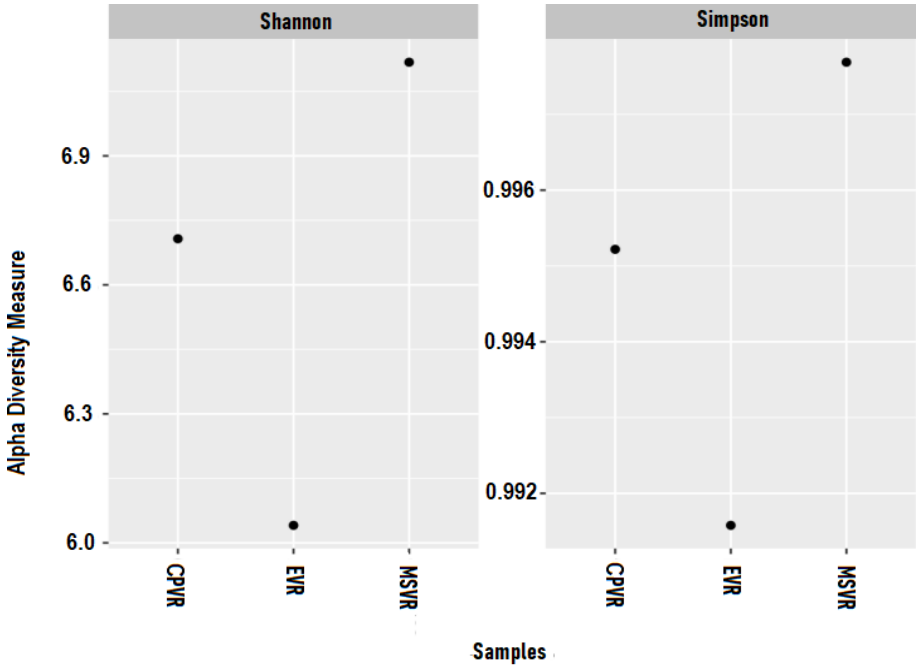


Fig 7.3.16: Alpha (Shannon and Simpson) diversity of bacterial communities in the vermireactors

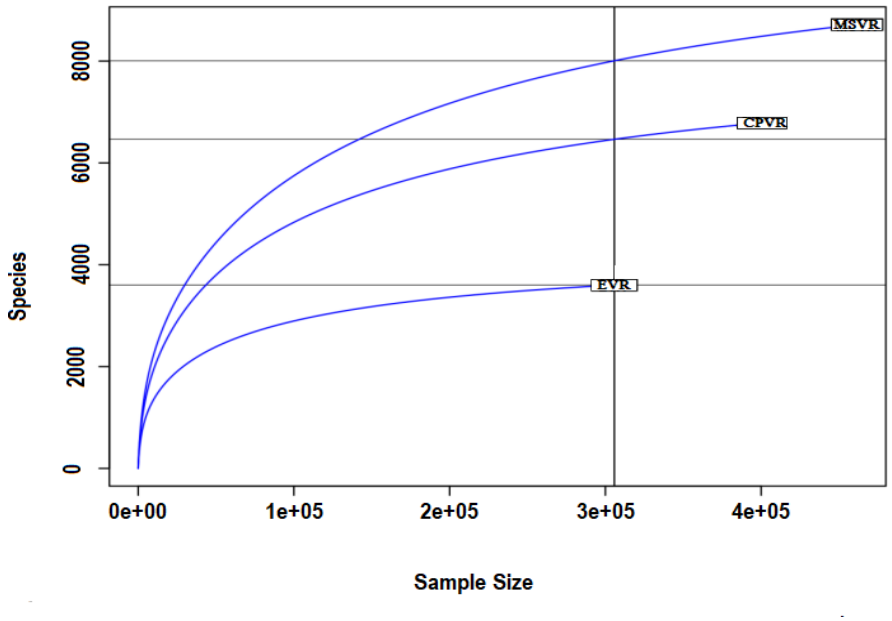
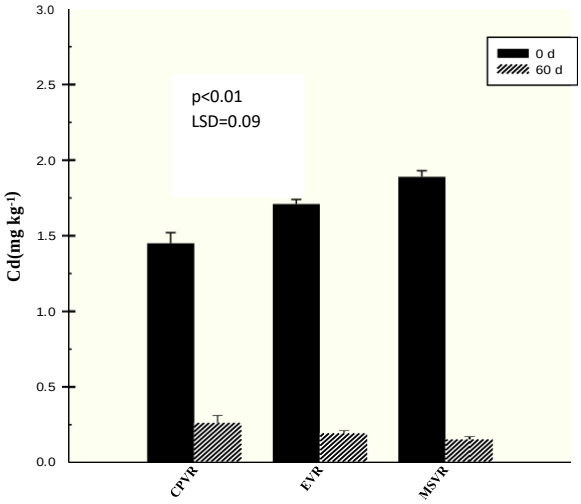
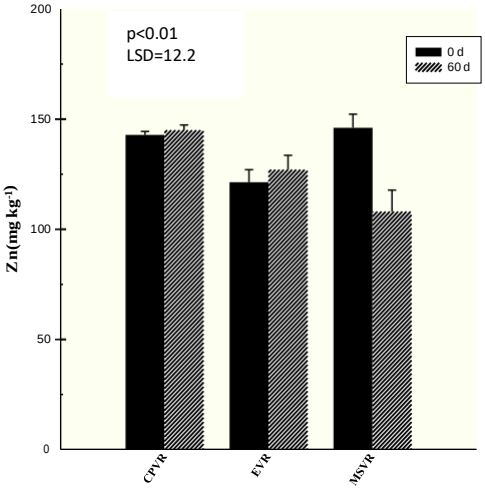


Fig 7.3.17: Rarefaction curves estimating the species richness in the vermireactors

Impact of vermireactors on metal bioavailability

The concentration of heavy metals (Cd, Zn, and Mn) was estimated in the feedstock of the different vermireactors at initial and final (i.e., 60 d) stages **(Figure.7.3.18 (a),(b),and(c))**. Among the studied heavy metals, Cd is a non-essential toxic element while Zn and Mn are essential elements but are toxic at high concentrations (Brady, 1995). Bioavailability of all three metals substantially decreased in the feedstock in all the vermireactors after 60 days of incubation. However, the extent of metal reduction was remarkable in the MSVR system **(Figure.7.3.18 (a))**. Cd availability decreased by 12.6 folds in the MSVR feedstock followed by EVR feedstock (9 folds). Similarly, Zn concentration significantly lowered in the MSVR feedstock by 1.35 folds. In contrast, slight increment in Zn levels was recorded in the CPVR and EVR feedstocks **(Figure 7.3.18 (b)**. The Mn concentration considerably reduced in the all the feedstocks and at the end of 60 d the Mn level in the reactors was in the order: MSVR= CPVR> EVR (p for treatment < 0.01; LSD = 0.27) **(Figure.7.3.18 (c))**. Interestingly, Mn reduction was highest in the EVR, which may be due to rapid leaching of the element under low pH condition (Liu et al., 2020). Overall, the results imply that metal removal efficiency, in particular Cd, of earthworms was greater in the MSVR as compared to CPVR and EVR. As such, feedstock characteristics and vermicomposting condition strongly influence metal detoxification of *Eisenia fetida* [31].





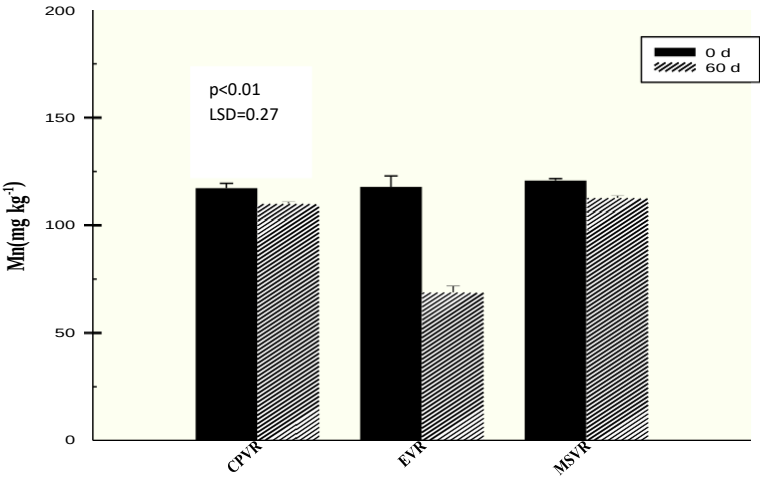


Fig.7.3.18. Temporal variation in (a) Cd(mg/Kg),(b) Zn(mg/Kg), and (c) Mn(mg/Kg) in the feedstocks under the composting and vermicomposting system Error bars represent the standard deviation; LSD means Least Significant Difference.

7.9.5 Impacts of vermireactor-mediated vermicomposts on seed vigor

The effectiveness of any plant growth promoting agent can be authentically evaluated through seed germination test [29,30]. Therefore, the impact of vermireactor-mediated vermicomposts on the germination profiles ofgreen gram (*Vignaradiata*) seeds was assessed. The data on germination index (GI), relative shoot and root germination (RShG and RRG), and relative seed germination (RSG) percentages are presented in **Table 7.1**.In general, the seeds germinated within 3 days under all the three treatments; therefore, the RSG after 5 days was more or less similar under all three treatments **Table 7.1**The significant differences between treatments were highly pronounced for RRG, RShG, and GI. The RRG was highest for MSVR vermicompost treated seeds followed by EVR and CPVR ($p < 0.001$; LSD = 5.894; **Table 7.1**).Similarly, the RShG of the seeds under different treatments was highest for the MSVR vermicompost followed by CPVR ($p < 0.001$; LSD = 2.669; **Table 7.1**). However, the GI of the green gram seeds after 5 days of incubation under different treatments was in the order: CPVR = MSVR > EVR ($p < 0.01$; LSD = 16.068; **Table 7.1**). Hence, the results of the seed germination assay strongly justified that the earthworm-induced hormonal and enzymatic activation was most prudent in the MSVR vermicompost than the others, which in turn, augmented cell development of the green gram seeds. Induction of useful hormones (cytokinins, kinetins, abscisic acid, etc.) in the vermicomposts is caused by the humic substances during feedstock decomposition [29,31]. Pramanik (2010) also reported that secretory function of earthworms induces the development and activation of hydrolytic enzymes.

Table 7.1: Comparison between the composted and vermicomposted feedstock extracts on relative root (RRG), shoot growth (RShG), relative seed germination (RSG), andgermination index (GI) ofgreen gram (*Vigna radiata*)

	Relative root growth %(RRG)	Relative shoot growth % (RShG)	Germination Index %(GI)
CPVR	122.73±10.11	156.09 ± 3.44	91.00 ± 5.59
EVR	142.46±5.47	140.17 ± 2.35	48.67 ± 1.18
MSVR	205.52 ± 4.92	205.44 ± 3.83	75.91 ± 2.71
p(treatment)	0.000	0.000	0.009
LSDtreatment	5.894	2.669	16.068

7.10 Conclusions

This study demonstrated that mechanized vermicomposting system can be beneficial to produce enriched vermicompost from biodegradable solid wastes within a short time period. The benefit of the MSVR was evidenced in terms of nutrient (N, P, and K) augmentation, organic C stabilization, and microbial activity. The earthworm fecundity was more pronounced in the MSVR than the CPVR and EVR; which probably facilitated the overall enrichment of the MSVR end-product. The microbial community structure positively shifted towards species enrichment. In particular, the proliferation of rare species was greatly promoted in the MSVR as evidenced from 16S rRNA gene sequencing based metagenomic analysis. Moreover, the quality of the vermicompost was proved to be better under MSVR system than the CPVR and EVR in regard to metal removal and promotion of vigorous growth potential of the germinated green gram seeds. However, the practical and economic utility of the evaluated technology warrants more extensive studies for developing it into a viable intellectual property in near future.

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