

ORIGINAL ARTICLE

Quality Evaluation of Vermicompost at various Phases of farm Waste Composting and during Storage

Manjul Gupta^{a,b*}, Shikha^b, Pankaj Kumar Srivastava^a, Shri Krishna Tewari^a

^aCSIR – National Botanical Research Institute, Rana Pratap Marg, Lucknow, India - 226001.

^bSchool of Environmental Sciences, Babasaheb Bhimrao Ambedkar University Vidya Vihar, Rae Bareilly Road, Lucknow – 226025.

E-mail: manjulnbri@gmail.com

ABSTRACT

The present investigation was aimed to evaluate the microbial population and enzyme activities during different phases of farm waste vermicomposting and during storage phase. Initial thermophilic decomposition of waste load using cow-dung slurry was done in the decomposing bed and after this transferred in to vermibed. The maximum values of all enzymatic activities were found at vermibed phase than in decomposing phase leading to the conversion of farm waste into value added product. Similarly, the evaluation of microbial population like bacteria, fungi, Actinomycetes, Bacillus, was found at vermibed phase as compared to decomposing phase. However, in final product (vermicompost) maximum value of all enzymatic activities and microbial population were found at 5 day afterwards their activity showed a declining trend with the time (15, 30 days). This study clearly indicates that earthworms (at vermibed phase) strongly modify the microbial population and enzymatic activities during vermicomposting of farm waste and suggest that the final product (vermicompost) should be utilized for its manurial purposes maximum within a month as the fertility status starts declining afterwards.

Keywords: Decomposition, earthworm, microbial biomass, organic waste, enzyme activity

Received 19/01/2014 Accepted 25/02/2014

©2014 Society of Education, India

How to cite this article:

Manjul Gupta, Shikha, Pankaj K. S., Shri K. T. Quality Evaluation of Vermicompost at various Phases of farm Waste Composting and during Storage. Adv. Biores., Vol 5 [1] March 2014: 56-63. DOI: 10.15515/abr.0976-4585.5.56-63

INTRODUCTION

Vermicomposting is a bio-oxidative process in which earthworms interact intensively with microorganisms thereby, accelerating the stabilization of organic matter and eventually modifying its physical and biochemical properties [1]. It is considered a preferred method for municipalities and industries to recycle a variety of organic by products in order to apply them as soil conditioners and amendments [2]. One of the most important factors affecting the use of vermicompost for agricultural practices is its degree of maturity and stability. If unstable or immature compost is applied it can induce anaerobic condition as the microorganisms utilize O₂ in the soil pores to break down the material [3] or may induce phytotoxicity due to the presence of organic acids during the early phase of the vermicomposting process. The seed germination index is a common biological method to evaluate the degree of maturity of compost. Physical parameters such as temperature, odour, colour, chemical parameters such as C/N ratio or humification index and biochemical parameters have been studied to characterize compost maturity [4]. The evaluation of enzymatic activities (EA) and microbial population during composting can also reflect the dynamics of the composting process in terms of the decomposition of organic matter [5], nitrogen transformations and may provide information about the stability and maturity of composted product [6]. The enzymes involved in the composting process includes cellulases, which depolymerise cellulose, β-glucosidases which hydrolyse glucosides, proteases and urease involved in N mineralization, phosphatases and aryl-sulphatase that remove phosphate and sulphate groups. Therefore, EA should indicate the ability of compost to degrade a wide range of common organic substrates. The pattern of microbial communities along the process may also provide valuable information regarding the evolution of the process, the rate of biodegradation and, finally, the maturity of

the product [7]. Microbial activity is achieved through the secretion of the enzymes that are responsible for the hydrolysis of complex macromolecules that constitute the organic wastes. As a consequence of this activity, simple water-soluble compounds that support microbial growth are released favouring the continuity of the process.

Keeping in view the above facts, the present study was conducted to assess the evolution of some important enzymatic activities due to prevalence of microbial population, during the different phases of vermicomposting and the effects of vermicompost aging, especially patterns of changes in microbial community and enzyme activities, because these parameters can control the quality of the vermicompost to be utilized as manure.

MATERIALS AND METHODS

Experiment description

The experiment was carried out at the Biomass Research Centre (BRC) of CSIR-National Botanical Research Institute (80° 45' to 80° 53'E and 26° 40' to 26° 45' N at an elevation of 129m above the sea level) Lucknow, India. The vermicompost was prepared at the same research centre by using farm waste (consisted of mixed leaf litter of *Acacia nilotica* Delile sub sp. *indica* (Benth.) Brenan, *Eucalyptus tereticornis* Sm., *Albizia lebbek* (L.) Benth., *Azadirachta indica* A. Juss., *Leucaena leucocephala* (Lam.) de Wit., *Terminalia arjuna* Wight and Arn., *Prosopis juliflora* (Swartz) DC., *Dahlia pinnata* Cav., *Polianthes tuberosa* L., *Bougainvillea glabra* Choisy, *Plumeria rubra*, *Cassia fistula* L.) as described by Srivastava *et al.* [2]. Farm waste was thermophilically composted for one month initially with 1:10 cow-dung slurry and manual turning every 10d to reduce pathogen populations (decomposing phase). The composted waste, adjusted to 60%–70% moisture, was placed into vermibeds (vermibed phase). *Eisenia fetida* culture (at 2.5 kg earthworms m² per bed) was added for vermicomposting for the next 45 d [2]. Vermicompost was obtained by filtering the fully decomposed black granular material from the vermibed using 2-3 mm mesh/sieve. The filtered (freshly harvested) moist samples were stored in sealed polythene bags at 4°C for enzymatic and microbial analysis. Five sub samples of composting materials were taken from decomposing bed on days 5, 15, 30 (coded as T₁, T₂ and T₃) and/or from vermibed (after transferring the half-decomposed waste load from decomposing bed) on days 5, 15, 30 and 45 (coded as T₄, T₅ and T₆, T₇) and mixed thoroughly prior to enzymatic and microbiological analysis. The final product vermicompost samples were also taken at 5, 15 and 30 days (coded as V₁, V₂ and V₃) to know the shelf life of compost to be utilized for manurial purpose.

Soil enzymatic activities

The enzymatic activities { β -glucosidase (GA), alkaline phosphatase (AP), dehydrogenase (DHA), protease (PA) cellulase (CeA) and catalase (CA)} were analyzed by using the methods given earlier by Srivastava *et al.* [2].

Isolation and enumeration of viable microbes

Viable microbes (Bacteria, Phosphate solubilizing bacteria, Nitrogen fixing bacteria, Pseudomonas, Bacillus, Fungi, Actinomycetes, Phosphate solubilizing fungi) present in the samples were determined by standard dilution plating procedure as described earlier by Srivastava *et al.* [2].

RESULTS

Enzymatic activities during different phase of vermicomposting

Changes observed in enzymes activities throughout vermicomposting process has been presented in Figure (1) A-F. The maximum values of all enzymatic activities were observed at T₅ phase. With the advancement of the process the cellulase and catalase activities in vermicomposting were found to increase at T₅ phase and reached the peak value of 0.452 μg reducing sugar g⁻¹ h⁻¹ (Figure 1, A) and 0.751 mmol H₂O₂ g⁻¹ h⁻¹ (Figure 1, B), respectively. Similarly, dehydrogenase and β -glucosidase activities also reached to peak values of 1.6 μg TPF g⁻¹h⁻¹ (Figure 1,C) and 228 μg p-Nitrophenol g⁻¹h⁻¹ (Figure 1,D) on T₅ phase, respectively. Similar pattern was observed in the case of alkaline phosphatase activity (Figure 1, E).

All the enzymatic activities were found to decrease after T₅ phase as composting process proceeded. The protease activity increased at the beginning of vermibed phase and reached the peak value of 80 μg amino-acid g⁻¹h⁻¹ at T₅ phase followed by a gradual decline from T₆ to T₇ phase (72 μg amino-acid g⁻¹h⁻¹) (Figure 1,F). However, in the final product (vermicompost) maximum value were recorded for all enzymatic activities as recorded on 5 day of sampling and afterwards their activity showed a declining trend (Figure 1, A-F)

Pattern of microbial population during the different phase of vermicomposting

Change observed in microbial community composition throughout the vermicomposting process has been summarized in Figure 2 (A-C). The population of free-living nitrogen fixing bacteria showed maximum values from the samples taken at phase T₄ which got stabilized afterwards. Correspondingly, the fungal and bacterial populations were found to be highest at T₄ phase of composting and afterwards their activity tends to be stabilized (Figure 2, B).

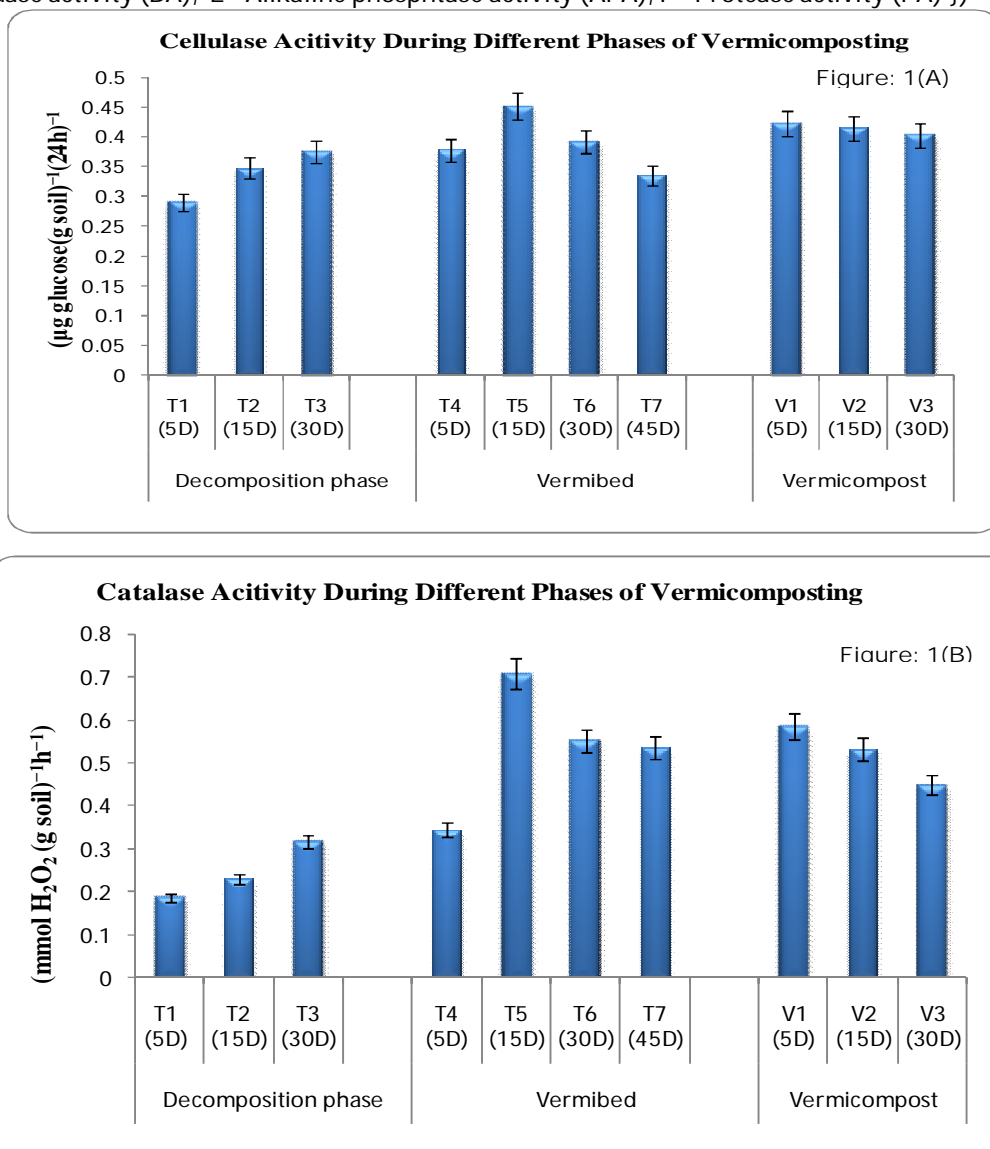
Similarly, the population of phosphate solubilizing bacteria was found maximum (3.5×10^6 cfu ml⁻¹) at phases T₄ and T₅ while the population of fungi increased gradually (from T₁ to T₃) as vermicomposting process proceeds and the maximum population (5.5×10^6 cfu ml⁻¹) was found at T₄ phase of vermicomposting (Figure 2, B). Likewise, the community of *Bacillus* and *Pseudomonas* were observed highest 2.5×10^6 cfu ml⁻¹, and 4.5×10^6 cfu ml⁻¹, respectively at phase T₄. However, in final product (vermicompost) the overall microbial population was found highest on 5th day and afterwards their population declined (Figure 2, C).

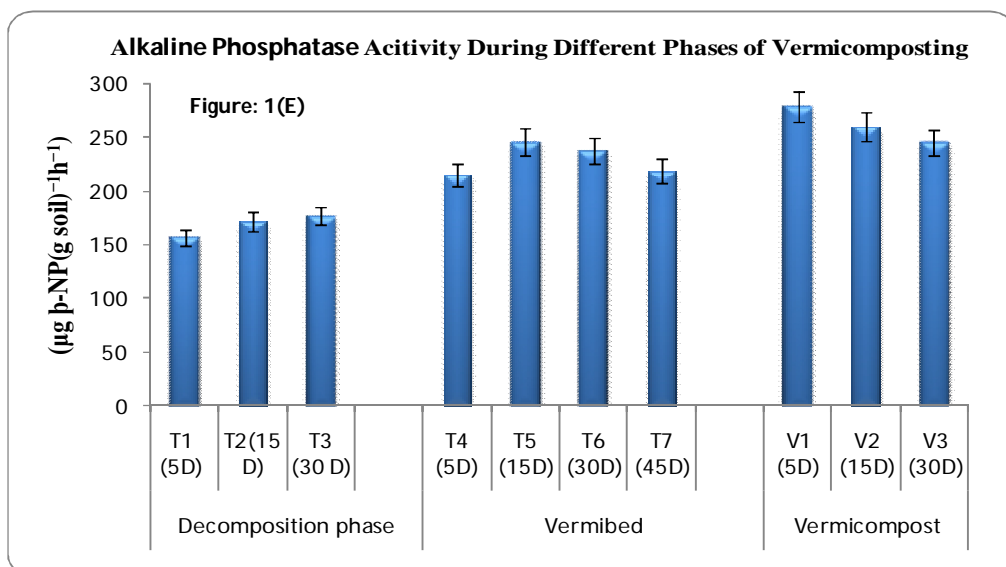
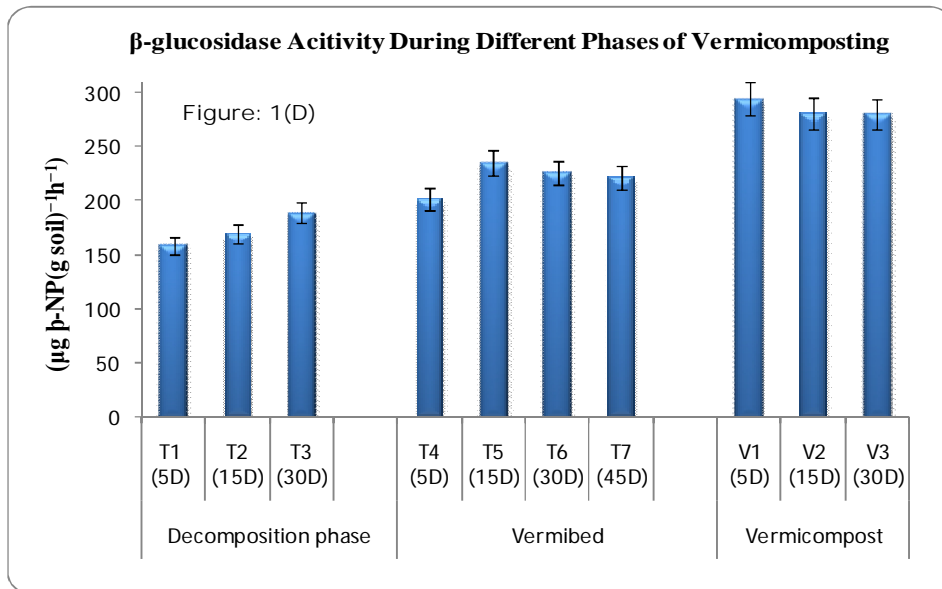
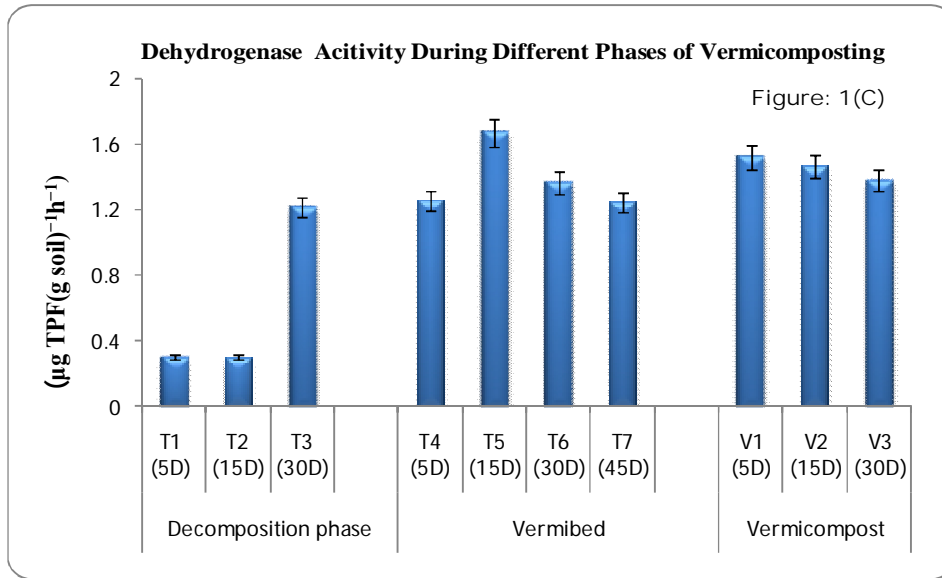
DISCUSSION

The results of the present study showed that once earthworms (vermibed phase) have entered the organic waste, the process of vermicomposting which is mainly characterized by microbial activity experience a continual increase as indicated by enzymatic activities. All the enzymatic activities measured in the present study were found to increase during vermibed phase of composting in accordance with the dynamics of the microbial community, which indicates that the indigenous microorganisms were able to synthesize the enzymes required for degradation and hydrolysis of various organic compounds, including complex substances [8]. The changes in the activities of enzymes; cellulase, β -glucosidase, protease and dehydrogenase which are responsible for hydrolysis of cellulose, hemicelluloses and proteins, respectively, gives the valuable information related to the dynamics of important nutritional elements like C, N or P and contributes to a better understanding of the transformations that take place during composting. Dehydrogenase activity was chosen as an index of microbiological activity because it refers to a group of intracellular enzymes which catalyse the oxidation of soil organic matter [9]. The high DHA activity in vermibed might have been the result of high microbial activity due to the high water-soluble carbon (WSC) and fulvic acid (FA) contents. The β -glucosidase is another one of the key enzymes which govern the carbon cycle. It hydrolyses reducing terminations of β -D-glucose chains to give β -glucose [10]. Its activity is therefore indicative of the presence of these terminations, which come from the labile organic matter. Thus, those composting phases characterized by a higher availability of such compounds should be associated to greater β -glucosidase activity. The increase in the β -glucosidase activity in the vermibed phase might be due to the presence of carbon compounds derived from the cellulolytic and hemi-cellulolytic activities, which are primarily achieved during the final thermophilic phase and the cooling phase that precedes the maturation [11]. Phosphatase catalyses the hydrolysis of organic phosphorus compounds to different inorganic forms which plants can metabolize. This enzymatic activity plays critical roles in P cycles and it is considered a general microbial indicator. In this study, higher levels of phosphatase were observed, which might be due to the abundance of organic phosphorus compounds that characterize these biosolids [12]. Protease activity is closely related to the N cycle and catalyses the hydrolysis of proteins, increased sharply at the beginning of the process, most likely because of the suitable availability of oligo and polypeptides in the initial mixture. Proteases act on proteins and polypeptides degradation and they can be considered good indicators of organic matter decomposition on account of their extreme dependence on substrate availability, [13]. The cellulase activity that catalyses the hydrolysis of cellulose to D-glucose is dependent on the types of cellulolytic microorganisms present in the mixture. In general, the higher activity of cellulase enzymes in vermibed indicated that microorganisms are able to effectively and rapidly synthesize enzymes required for the degradation of polymeric substances such as cellulose, hemi-cellulose and lignin. Decomposting phase was shown relatively less enzymatic activities as compared to vermibed, might be attributed to the slow and incomplete stabilization of organic matter. Microbial community also play a key role in composting process and appearance of some microorganisms reflects the quality of maturing compost [14]. In the present study, higher enzymatic activities was observed at vermibed phase as compared than in decomposting phase mainly due to the presence of earthworms and aerobic heterotrophic microbial population. Higher population of fungus and bacteria in vermibed phase has been attributed to the presence of easily degradable organic compounds. Enhanced fungal population after gut transit was also found by Aira *et al.* [15] after vermicomposting of pig slurry with *Eisenia fetida*. Fungi are actively involved in the decomposition of cellulose, hemicelluloses and lignin present in the organic matter which was reflected by their population at different phases of composting. The increase of microbial population may be caused by congenial condition for the growth of microbes within the worm digestive tract and by

the ingestion of nutrient rich organic wastes which provide energy and also act as a substrate for the growth of microorganisms as reported by Tiwari *et al.* [16]. The evolution of autotrophic nitrifying bacteria also showed an increment in population until the samples taken at T₆ sample. In fact, their sequential action produces NH₄⁺ which is oxidized to NO₃⁻ N by autotrophic nitrifying bacteria (first by ammonium oxidizing bacteria *Nitrosomonas* spp., and after by nitrite oxidizing bacteria *Nitrobacter* spp.). Higher population of phosphate solubilizing microorganisms has been attributed to the conversion of complex form of phosphorus to available forms which include altering the solubility of inorganic compounds to the ultimate soluble form by production of acids and H₂S under aerobic and anaerobic conditions and by mineralizing organic compounds, with the release of inorganic phosphate [17]. The progressive decrease in microbial population observed in vermicompost (final product) might have occurred due to lack of earthworm population at this phase. Aira *et al.* [18], reported that once earthworms have left the organic waste, the process of aging of vermicompost is mainly characterized by microbial processes, which experience a continual decrease as indicated by enzymatic activities. More specifically, the presence of *Eisenia fetida* first promoted an increase and then a decrease in both the microbial biomass and activity after 8 weeks during vermicomposting of pig manure. Likewise, the absence of degradable organic compounds (available substrate) in vermicompost might have decreased the enzyme synthesis; as substrate decreased, the enzymatic activities decreased as well [15].

Figure 1: Changes observed in enzymatic activities at different phases of vermicomposting and during storage. (A= Cellulase activity (CeA), B= Catalase activity (CA), C= Dehydrogenase activity (DHA), D= β -glucosidase activity (BA), E= Alkaline phosphatase activity (APA), F= Protease activity (PA))





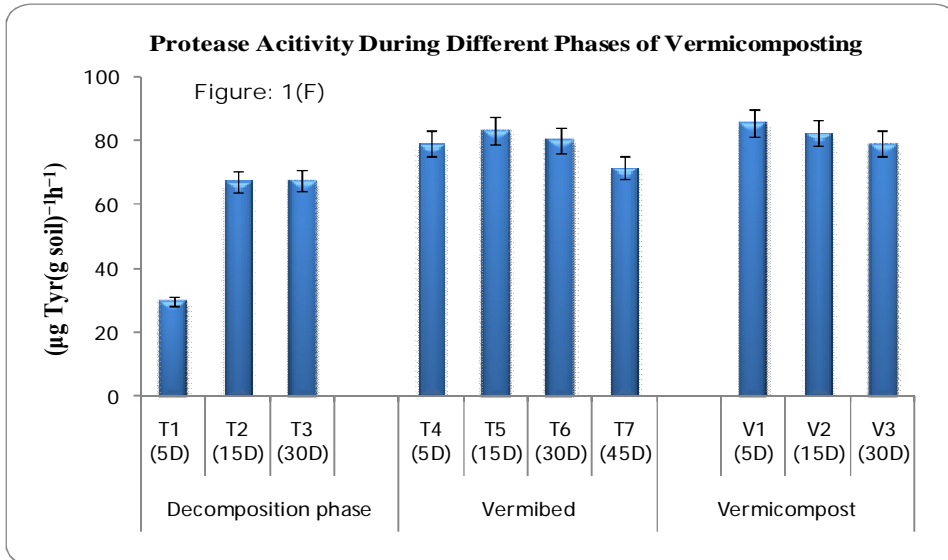
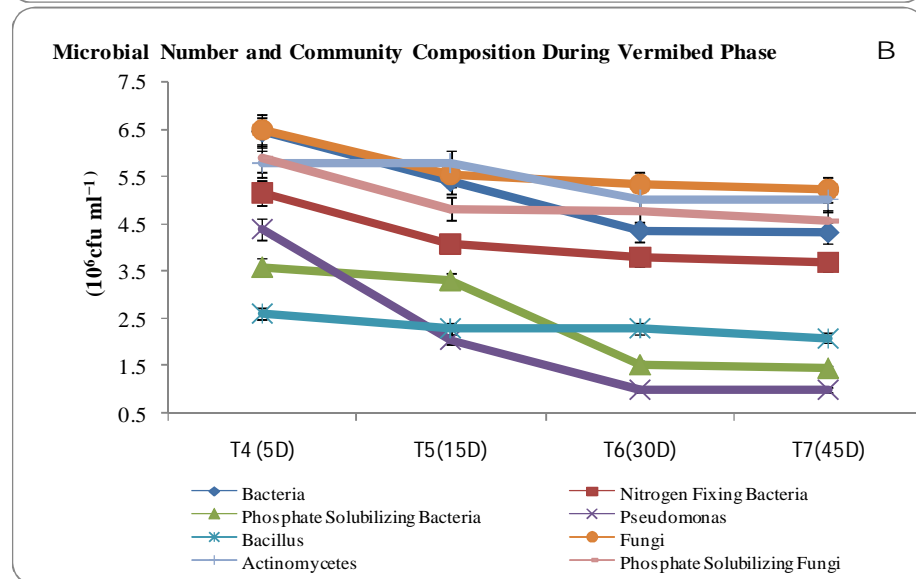
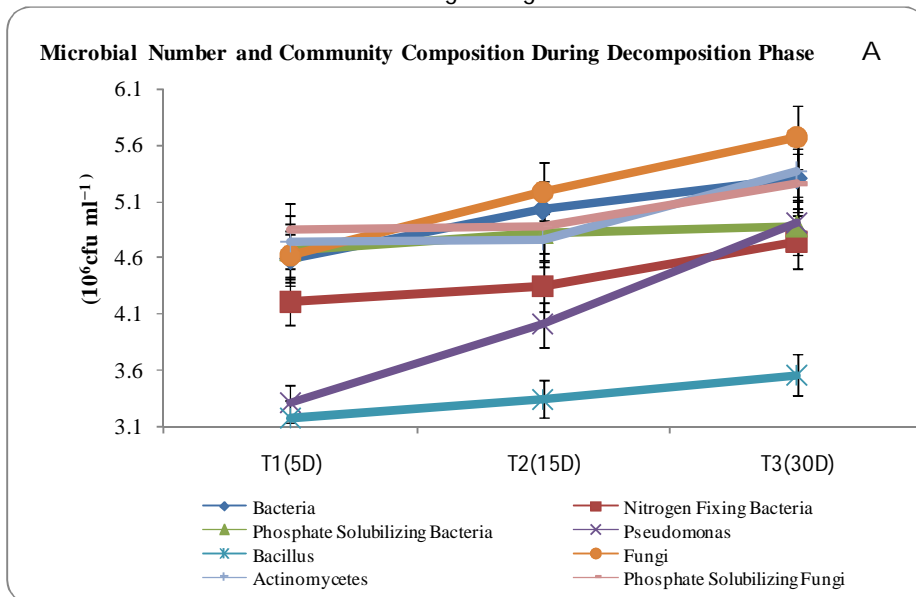
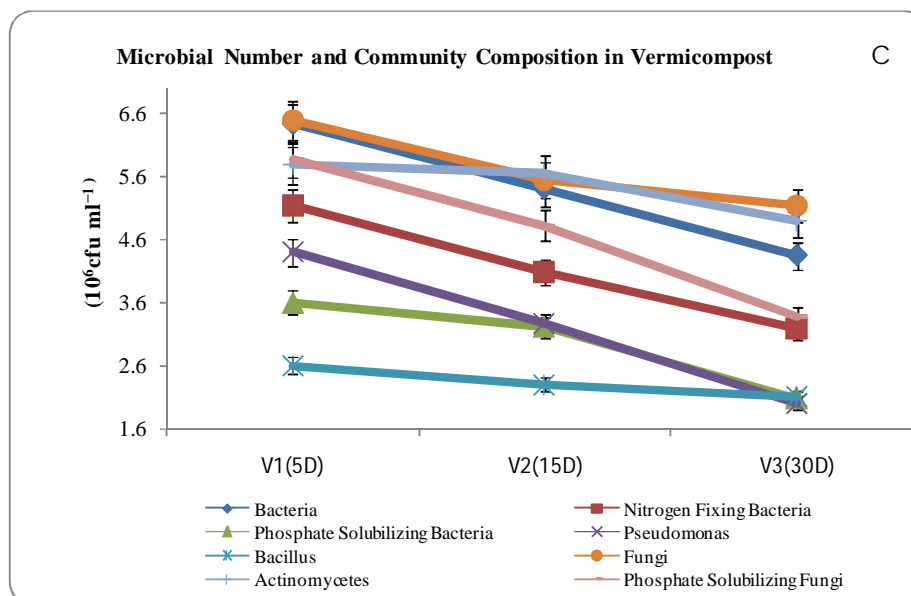


Figure 2: (A-C): Pattern of microbial population during the different phases of vermicomposting and during storage.





CONCLUSION

According to the results, enzymatic activities (EA) and microbial community composition at different phase of vermicomposting could be used to characterize composting process and that the achievement of a stable EA could be a reliable index of compost stability. The conclusion drawn from the present study was that vermicompost can be considered as one of the best organic manure because it not only provide the available nutrient to soil but also enhance the soil fertility by adding beneficial microbes to soils. It can be suggested that the vermicompost should be utilized for its manurial purposes maximum within a month and preferably within 15 days, as the fertility status, its viability and microbial population of harvested vermicompost starts declining afterwards.

ACKNOWLEDGEMENTS

Authors are grateful to Director, CSIR – National Botanical Research Institute, Lucknow, India and Council of Scientific Industrial Research (CSIR), India for providing institutional and financial support under the CSIR Network Project, respectively.

REFERENCES

- Dominguez, J., (2004). State of the art and new perspectives in vermicomposting research. (Eds. Edwards, C.A.). Earthworm ecology, CRC Press, Boca Raton, pp. 401–425.
- Srivastava, P.K., Singh, P.C., Gupta, M., Sinha, A., Vaish, A., Shukla, A., Singh, N., & Tewari, S.K., (2011). Influence of earthworm culture on fertilization potential and biological activities of vermicomposts prepared from different plant wastes. *J. Plant Nutr. Soil Sci.* 174: 420–429.
- Mathur, S.P., Owen, G., Dinel, H., & Schnitzer, M., (1993). Determination of compost biomaturity I. Literature review; *Biology Agri. Horti.* 10, 65–85.
- Mondini, C., Fornasier, F., & Sinicco, T., (2004). Enzymatic activity as a parameter for the characterization of the composting process. *Soil Biol. Biochem.* 36: 1587–1594.
- De La Horra, A.M., Defrieri, R., Jimenez, M.P., & Palma, R.M., (2005). Evolution of alkaline phosphatase and protease activities, total organic carbon and CO₂ evolved during composting. *Agrochimica.* 49, 22–28.
- Cunha-Queda, A.C., Vallini, G., Agnolucci, M., Coelho, C.A., Campos, L., & Bruno de Sousa, R., (2002). Microbiological and chemical characterization of composts at different levels of maturity, with evaluation of phytotoxicity and enzymatic activities (Eds. Insam, H., Riddech, N. and Klammer, S.) *Microbiology of Composting.* Springer-Verlag, Berlin Heidelberg, Germany, pp. 346–355.
- Ryckeboer, J., Mergaert, J., Coosemans, J., Deprins, K., & Swings, J., (2003). Microbiological aspects of biowaste during composting in a monitored compost bin. *J Appl. Microbiol.* 94: 127–137.
- Margesin, R., Cimadom, J., Schinner, F., (2006). Biological activity during composting of sewage sludge at low temperatures. *Int. Biodeter. Biodegr.* 55: 88–92
- Nannipieri, P., Grego, S., & Ceccanti, B., (1990). Ecological significance of the biological activity in soils. (Eds. Bollag, J.M. and Stotzky, G.) *Soil Biochemistry*, Marcel Dekker, New York, pp. 293–355.
- Nannipieri, P., Kandeler, E., & Ruggiero, P., (2002). Enzyme activities and microbiological and biochemical processes in soil (Eds. Burns, R.G., and Dick R.P.) *Enzymes in the Environment. Activity, Ecology and Applications*, Marcel Dekker, New York, pp. 1–33.

11. Yu, H., Zeng, G., Huang, H., Xi, X., Wang, R., Huang, D., Huang, G., & Li, J., (2007). Microbial community succession and lingo-cellulose degradation during agricultural waste composting. *Biodegradation*. 18: 793–802.
12. Benitez, E., Sainz, H., Nogales, R., (2005). Hydrolytic enzyme activities of extracted humic substances during the vermicomposting of a lignocellulosic olive waste. *Bioresour. Technol.* 96: 785–790.
13. Lazcano, C., Gomez-Brandon, M., & Dominguez, J., (2008). Comparison of the effectiveness of composting and vermicomposting for the biological stabilization of cattle manure. *Chemosphere*. 72: 1013–1019.
14. Ishii, K., & Takii, S., (2003). Comparison of microbial communities in four different composting processes as evaluated by denaturing gradient gel electrophoresis. *J Appl. Microbiol.* 95: 109–119.
15. Aira, M., Monroy, F., & Dominguez, J., (2006). Changes in microbial biomass and microbial activity of pig slurry after the transit through the gut of the earthworm *Eudrilus eugeniae* Kinberg. *Biol Fertil Soils*. 42: 371–376.
16. Tiwari, S.C., Tiwari, B.K., & Mishra, R.R., (1989). Microbial populations, enzyme activities and nitrogen - phosphorous - potassium enrichment in earthworm casts and in the surrounding soil of a pineapple plantation. *Biol. Fertil. Soils*. 8: 178–182.
17. Rasal, P.H., Kalbhor, H.B., Shingte, V.V., & Patil, P.L., (1988). Development of Technology for Rapid Composting and Enrichment. *Biofertilizers: Potentialities Problems* (Eds. Sen, SP., and Palit P.) Plant Physiology Forum and Naya Prakash, Calcutta, India, pp. 255–258.
18. Aira, M., Monroy, F., & Dominguez, J., (2007). Earthworms strongly modify microbial biomass and activity triggering enzymatic activities during vermicomposting independently of the application rates of pig slurry. *Sci. Total Environ.* 385: 252–261.