Optimizing microbial activity during vermicomposting with different earthworm densities

Abstract

This study was performed to explore impact of earthworm densities with addition of biofertilizer for optimizing microbial activity during vermicomposting. Equal numbers of nitrogen fixing bacteria (NFB) and phosphate solubilising bacteria (PSB) were inoculated in the vermicomposting system which was incubated separately with 0, 10, 20, 30, 40 and 50 nos. of epigamic earthworm *Eisenia foetida* per kg of substrate. The study revealed the nos. of inoculated microorganisms *viz.* NFB and PSB in the substrates to increase with increase in earthworm population up to a certain range. The values reached almost stagnation when the stocking density of earthworms was increased beyond 30 nos. kg^{-1}. Between the two kinds of inoculated microflora, however, the trend of stagnation became more prominent for NFB than PSB. The CFU of NFB and PSB in earthworm guts also showed a similar trend. This increased CFU of the microflora was reflected in the MBC values of the substrates where incubation with 30-50 earthworms per kg substrate showed highest ranges of MBC values. Similar was the trend for microbial respiration also. CEC, easily oxidisable organic carbon and total organic carbon values were used as indices of composting in the present study. All these values showed that the rate of vermicomposting showed a stagnating trend when the earthworm density was increased beyond 30 nos. kg^{-1}.

Keywords: Earthworm, Vermicompost, Phosphate solubilising microorganisms, Nitrogen fixing bacteria, Cation exchange capacity,Microbial biomass carbon, Microbial respiration, Total organic carbon, Microbial activity

Abbreviation:NFB Nitrogen fixing bacteria; PSB Phosphate solubilizing bacteria; PSM Phosphate solubilising microorganisms; CEC Cation exchange capacity; MBC Microbial biomass carbon; TOC Total organic carbon; CFU Colony forming unit

Introduction

It is well known that any composting process is carried out with the help of microorganisms [12]and the statement is true for vermicomposting also. Although earthworms tend to accelerate the vermicomposting process [7], it is the microorganisms in earthworm guts that actually play the key role in the decomposition of the organic waste materials to form vermicompost [11]. These microbes grow in the conducive environments of earthworm intestines [3] and the presence of earthworms in the composting system is likely to influence the population as well as the composition of microorganisms in the substrate and thus exert profound effect on decomposition of the wastes. In view of this background, an effort was made in this yard study to investigate the effect of earthworm population on microbial activity during vermicomposting. For getting a clear picture of microbial activity, the study was carried out in absence and presence of added biofertilizing microorganisms. It has been hoped that the study would generate some important information on standardization of earthworm population for optimizing the microbial activity during the course of vermicomposting.

Methodology

The study was carried out in yards using earthen pots. Organic wastes comprising of cow dung and straw at 1:1 ratio were used as the substrate. Two kg weight of the organic waste (OW) was taken in each of 21 nos. of pots and following treatments were used with 3 replicates for each.

Treatments

T_1 : Control (OW containing cow dung and straw at 1:1 ratio)

T_2 :OW + 10gPhosphate solubilizing bacteria (PSB) + 10gNitrogen fixing bacteria (NFB)

T_3 :OW + 10gPSB + 10gNFB + 10 nos. earthworms (EW)

T_4 :OW + 10gPSB + 10 g NFB + 20 nos. EW

T_5 :OW + 10gPSB + 10 g NFB + 30 nos. EW

T_6 :OW + 10gPSB + 10 g NFB + 40 nos. EW

T_7 :OW + 10gPSB + 10 g NFB + 50 nos. EW

PSB (*Pseudomonassp.*) NFB (*Azotobactersp.*) having 10×10^6 nos. g^{-1} populations, as procured from market, were inoculated in the substrates after 10 days of initial decomposition, after the primary thermophilic condition subsided. Applications of earthworms (*Eiseniafoetida*) with mean weight of 0.6 g for each were done after 5 days of inoculation of the microbes at different rates. An approximate moisture status of 40-50% was maintained in the substrates throughout the period of study.

The incubation was carried out for 60 days and samples were drawn from the substrates at 15 days intervals. While a part of each of the samples was dried in air for expressing the results on air-dry weight basis, the rest of the moist samples wasanalyzed for the following parameters:

At 15 days interval

i) Cation exchange capacity (CEC)

ii) Microbial Biomass Carbon (MBC)

iii) Microbial Respiration

After 30 days of incubation

i) Colony Forming Units (CFU) of NFB in the substrates

ii) CFU of PSB in the substrates

iii) CFU of NFB in earth worm guts

iv) CFU of PSB in earth worm guts

After 60 days of incubation:

i) Easily oxidisable organic carbon (EOOC)

ii) Total organic carbon (TOC)

Methods for analyses of all these parameters have been dealt in Sec. 3.

Analytical method

Cation exchange capacity (CEC) values of the samples were determined by following the method of Harada and Inoko [5]. In addition to these common properties, several other parameters were studied for different studies, depending on the needs of the experiments.

In each case, microbial biomass carbon (MBC) was estimated by following the chloroform fumigation method as described by Vance *et al*. [13]. Here two parts of the organic waste sample, each weighing 2g were taken separately in a 50ml beaker and a 250ml conical flask fitted with stopper. The beaker was placed in a vacuum desiccator containing a vial of 10g soda lime and a beaker containing 25ml ethanol free chloroform. The desiccator was then evacuated until the chloroform boiled for 2 minutes. The desiccator with the content was then incubated at 25^0C for 24 hrs. After fumigation, the beaker containing chloroformed organic waste sample was then transferred to a 250ml conical flask. Both the sets of fumigated and unfumigated samples were extracted with 100ml 0.5MK_2SO_4 after shaking for 30

minutes and then filtered. The filtrates were finally used for determination of organic carbon with the help of standard potassium dichromate in presence of strong acid mixture (H_2SO_4 :H_3PO_4 :: 2:1 V/V). The difference between fumigated and unfumigated organic carbon value was used to calculate the microbial biomass carbon using a calibration factor and expressed as µg of MBC g^{-1} of sample. Basal respiration was estimated by following a modified method of Alef [1]. For this purpose, 10g of the organic waste material was taken in 1 L conical flask and wetted to 50% of it's maximum water holding capacity. A vial containing 10ml of 0.5MNaOH was hanged inside the flask and the mouth of the flask was closed with a cork. The flask was incubated at 25^0C for 24 hrs. The CO_2 evolved during this period was determined by titrating the residual NaOH with 0.1MHCl. Easily oxidisable organic carbon of the organic wastes were determined by following the well-known wet digestion method of Walkley and Black [15]. Total organic carbon contents of the organic waste samples were determined by digesting the acidified ferrous ammonium sulphate treated samples at 100^0C for 2 h and then back titrating the residual strength of the solution by standard potassium dichromate.Jenser's agar medium [6] was used for counting the colony forming units of non-symbiotic nitrogen fixing bacteria. Pikovskaia'stricalcium phosphate agar medium was used for enumeration of phosphate solubilizing microorganisms. Three earthworms weighing 0.62g each were surface sterilized by keeping the earthworms in 0.1N$CdCl_2$solution. The worms were then washed in distilled water, dried in tissue paper, homogenized in 1.0ml of phosphate buffer and then centrifuged in 2000 rpm. The clear solution was considered as the whole body homogenate for gut microflora. Then 0.1ml of homogenized sample was transferred aseptically into different mediums as required for various estimations, using spread plate technique and incubated at 30 ± 2^0C in a incubator and observed for the growth after 48-72 h.

Results and Discussion

Microbial biomass carbon (MBC) indicates the gross presence of different microorganisms in any system [2]. The MBC values in the substrates under different treatments during the period of incubation have been presented in Table 1. As observed from the table, the MBC values were the minimum in the control treatment throughout the period of study. The values improved considerably on inoculation of two bio-fertilizing microorganisms assuming significant higher values during the end of the study. This was obviously due to gradual multiplication of these microorganisms in the organic matter rich substrates. Use of earthworms increased the MBC values in all the treatments and the

values showed an increasing trend with increments in the numbers of earthworms in the substrates. As has been discussed earlier, the earthworms, while feeding on the organic wastes, ingest the microorganisms residing there. These microorganisms multiply profusely in the congenial environment of earthworm guts [3] and help in vermicomposting. Hence such increments in MBC values of the substrates with increments in the population of earthworms were expected. However, maximum MBC values were observed in case of 30- 40 nos. of earthworms and the values declined slightly under 50 nos. of worms kg^{-1} treatment. Since the variations between the treatments having 30 and 40 worms kg^{-1} were not statistically significant, Introduction of 30 nos. of earthworm (*Eiseniafoetida)* at a total biomass of 18 g for each kg of substrate may be considered to be optimum for maintaining highest microbiological activity during vermicomposting.

Microbial respiration also indicates the activity of different microorganisms in a system [9]. And this has a close relationship with MBC values. As observed from table-2, microbial respiration was the lowest in the control treatment and increased on inoculation of two kinds of microorganisms and also increments in the earthworm population. Similar behaviour for MBC has also been reported earlier. The values were considerably higher during the initial period of incubation and declined in the later phase of the study. This behaviour was attributed to larger availability of easily decomposable organic matter during the initial period of study, oxidation of which liberated larger amount of CO_2 through microbial respiration [14]. The earthworm treatments resulted in higher respiration values over the no earthworm treatment during initial period of the study, possibly due to increased pace of composting. However, the values declined during the later period, as the pace of vermicomposting slowed down. The variations in microbial respiration among the earthworm treatments were not found to be statistically significant, in general.

During the course of decomposition, organic matter is gradually transformed into humus [8], releasing larger numbers of exchange sites and increasing, thereby, the cation exchange capacity (CEC). Hence, CEC may be considered as a major indicator of compost maturity [5], as effected by microbial activities. In the present study, CEC values of the substrates increased consistently with increase in incubation period (Table 3) under all the treatments, obviously owing to gradual humification of the organic materials. Inoculation of microorganisms helped to increase the CEC values over the control treatment, although the increments were not significant in most of the cases. Such benefits of use of biofertilizers in hastening the composting have been reported by Gaur and Singh [4] also. The

earthworm treatments, in general, showed higher CEC values over the no earthworm treatment, obviously owing to increased rate of decomposition under higher microbiological activity. However, the CEC values assumed almost stagnation in the earthworm treatments during the later period of study, probably due to their early humification. At the end of the incubation, however, there was no statistically significant variation among the treatments excepting with the control.

Colony Forming Units (CFU) of nitrogen fixing bacteria (NFB) and phosphate solubilizing Bacteria (PSB) in the substrates under different treatments at 30 days of incubation has been presented in figure-1. As observed from the figure, the CFU values for both the inoculated microorganisms increased with increase in the numbers of earthworms. However, the magnitudes of such increments became smaller as the number of worms exceeded 30 nos. kg^{-1}.Similar observations with regard to MBC and microbial respiration have been discussed earlier.

CFU of these two microorganisms *viz*. NFB and PSB in the earthworm intestines have been presented in figure-2. As observed from the figure, the nos. of two microorganisms increased rapidly up to 30 nos. kg^{-1}population of worms. The increments showed a retarding trend thereafter, although numerical values of microorganisms in earthworm guts increased with the increasing population of earthworms. This behaviour may be due to the fact that with the increments in the population of earthworms in the substrates, larger numbers of the microorganisms were being ingested by the worms. These microbes, after their multiplication in the earthworm guts, were being released back to the substrates through excreta thus increasing the microbial population in the substrates. Similar increments in MBC values of the substrates have been discussed earlier. These increased numbers of microorganisms were, in turn, ingested by the worms to increase gradually their population in earthworm guts.

Amount of easily oxidisable and total organic matter in different treatments were estimated at 60 days of incubation. Easily oxidisable organic carbon (EOOC) ranged between 6.00 and 8.16 percent in all the treatments and there was not much variation among the treatments. In case of total organic carbon (TOC), however, the difference was more prominent. The control treatment showed highest value of TOC after the completion of the incubation owing to lesser rate of decomposition of organic matter. Inoculation of two bio-fertilizing microorganisms tended to reduce the TOC value considerably by accelerating the decomposition. Such beneficial effects of nitrogen fixing bacteria (NFB) on reduction of the C : N ratio of the organic wastes has been discussed by Gaur and Singh [4], which helps to accelerate the decomposition. The importance of phosphate solubilising bacteria (PSB) in providing

better P nutrition to different microbes during vermicomposting has been shown by Saha and Chattopadhyay [10]. In the present study also, inoculations of these biofertilizers have been observed to increase the MBC and microbial respiration in the substrates (Tables-1,2). These beneficial activities of these microbes helped to accelerate the rate of decomposition of the organic wastes and thus reduced the amount of organic C in the composts. Inoculation of earthworms accelerated the composting process further, as discussed earlier. However, not much variation could be observed in TOC values under different earthworms treatments. As has been indicated by CEC values (Table-3), the vermicomposts under earthworm treatments tended to reach maturity before completion of the incubation period. This resulted in lowering of the differences in TOC values among the earthworm treatments at the later part of the incubation.

The study has shown inoculation of earthworms to increase the microbial population in the substrates and to accelerate, thereby, the pace of composing. This behaviour showed an increasing trend upto 30-40 earthworms showing a biomass range of 33 to 44 g earthworm kg^{-1} waste. After this range, the relative activities of microorganisms and the rate of composting appeared to be gradually stagnating. However, this study has referred to the biomass of earthworms at the time of their release in the substrates only. The increase in earthworm population during the period of incubation has not been considered in this investigation.

Conclusion

Earthworms harbour different kinds of microflora in their guts, which proliferate in the congenial environment there and help to degrade different organic wastes through vermicomposting. Hence, population of earthworms in the vermicomposting system is likely to play an important role in determining the occurrence and, thereby, the activity of the microorganisms. However, information on optimum stocking density of earthworms with regard to microbiological activity in the vermicomposting system is very limited. With this background, a yard study was carried out during the first phase of the work programme to study the effect of earthworm density on microbial population and, thereby, the magnitude of degradation of the waste under during vermicomposting. For getting a clear picture of microbial activity, equal numbers of nitrogen fixing bacteria (NFB) and phosphate solubilising bacteria (PSB) were inoculated in the vermicomposting system which was incubated separately with 0, 10, 20, 30, 40 and 50 nos. of epigeic earthworm *Eisenia foetida* per kg of substrate.

Forthrightly observations on cation exchange capacity (CEC), microbial biomass carbon (MBC), microbial respiration, easily oxidisable organic carbon and total organic carbon and midway observations on colony forming units (CFU) of NFB and PSB in the substrates and earthworm guts were taken for the study. The study revealed the nos. of inoculated microorganisms viz. NFB and PSB in the substrates to increase with increase in earthworm population up to a certain range. The values reached almost stagnation when the stocking density of earthworms was increased beyond 30 nos. kg^{-1}. Between the two kinds of inoculated microflora, however, the trend of stagnation became more prominent for NFB than PSB. The CFU of NFB and PSB in earthworm guts also showed a similar trend. This increased CFU of the microflora was reflected in the MBC values of the substrates where incubation with 30-50 earthworms per kg substrate showed highest ranges of MBC values. Similar was the trend for microbial respiration also. CEC, easily oxidisable organic carbon and total organic carbon values were used as indices of composting in the present study. All these values showed that the rate of vermicomposting showed a stagnating trend when the earthworm density was increased beyond 30 nos. kg^{-1}. This behaviour was probably due to attainment of saturation in microbial population and activity in the system when earthworm population was increased beyond this density.

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Figure Legends:

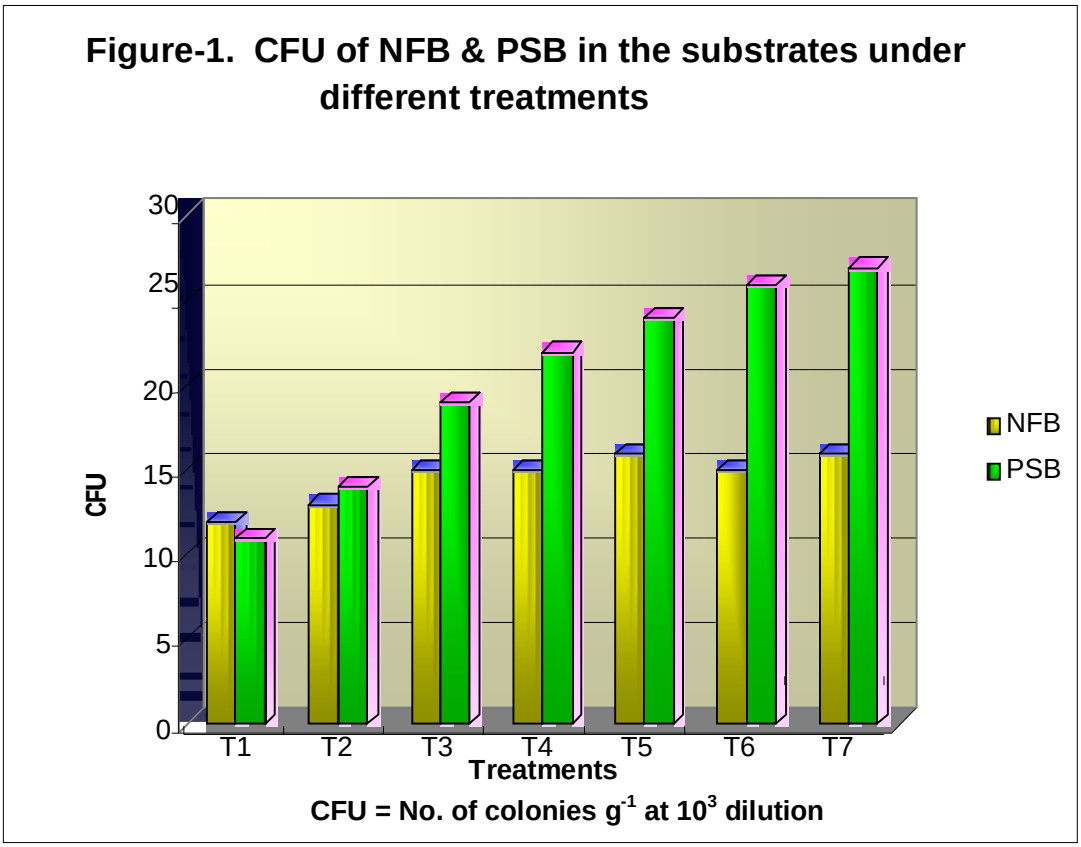

Figure 1: CFU of NFB and PSB in the substrates under different treatments

Figure 2: CFU of NFB and PSB in earthworm intestines under different treatments

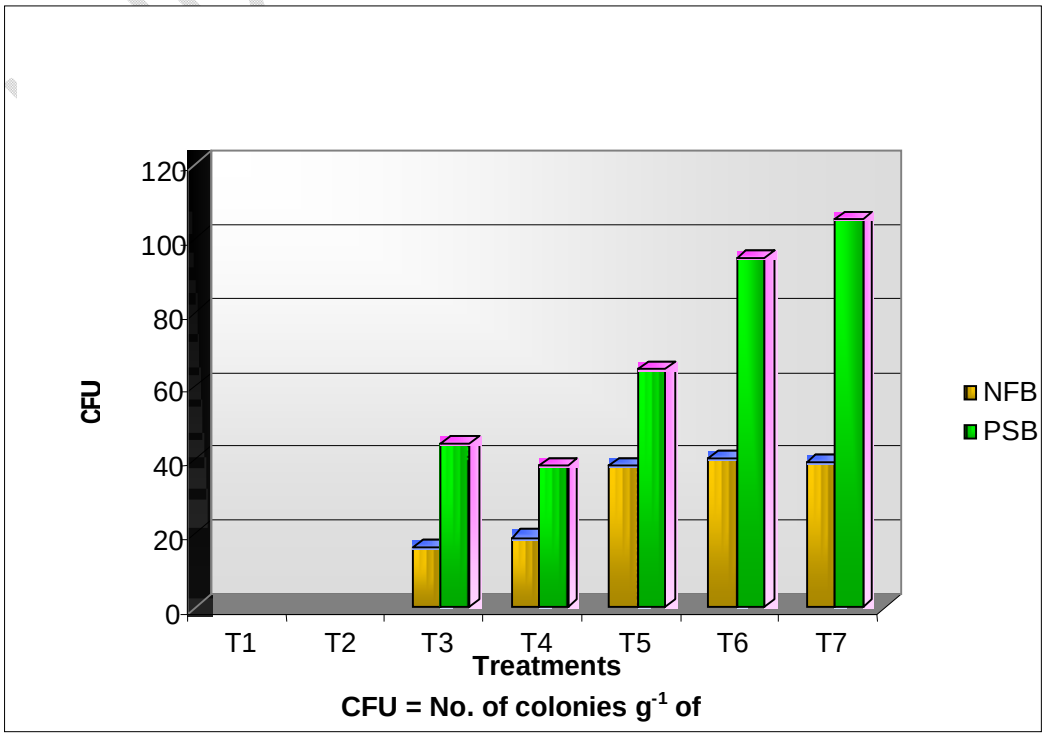

Table 1: MBC ($\mu g\ g^{-1}$) of air dry sample in different treatments during the period of incubation

Treatments	MBC at 15 days intervals	MBC at 30days intervals	MBC at 45 days intervals	MBC at 60 days intervals
T_1 = O.W. (500g CD +500gStraw)	324.84	408.56	371.56	345.36
T_2 = 10g P.S.B. + 10 g N.F.B+ O.W.	365.52	427.48	408.95	405.76
T_3 = 10g P.S.B. + 10 g N.F.B+ O.W+ 10 E.W	400.25	452.06	425.86	408.31
T_4 = 10g P.S.B. + 10 g N.F.B+ O.W+ 20 E.W	387.63	446.96	439.67	444.3
T_5 = 10g P.S.B. + 10 g N.F.B+ O.W+ 30 E.W	441.12	492.64	475.64	470.41
T_6 = 10g P.S.B. + 10 g N.F.B+ O.W+ 40 E.W	454.24	523.06	485.76	470.41
T_7 = 10g P.S.B. + 10 g N.F.B+ O.W+ 50 E.W	419.85	509.02	479.64	452.67
C. D.	**49.54**	**28.31**	**47.64**	**38.93**

T_1 : Control (OW containing cowdung and straw at 1:1 ratio); **T_2 :**OW + 10g phosphate solubilising bacteria (PSB) + 10g nitrogen fixing bacteria (NFB); **T_3 :**OW + 10gPSB + 10gNFB + 10 nos. earthworms (EW); **T_4 :** OW + 10g PSB + 10 g NFB + 20 nos. EW; **T_5 :** OW + 10g PSB + 10 g NFB + 30 nos. EW; **T_6 :** OW + 10g PSB + 10 g NFB + 40 nos. EW; **T_7 :**OW + 10gPSB + 10 g NFB + 50 nos. EW

Table 2: Microbial respiration (mg. CO_2 g^{-1} of air dry sample hr^{-1} at 25^{o} C) in different treatments during the period of incubation

Treatments	Microbial res. at 15 days intervals	Microbial res. at 30days intervals	Microbial res. at 45 days intervals	Microbial res. at 60 days intervals
T_1 = O.W. (500g CD +500gStraw)	1.22	0.98	0.63	0.48
T_2 = 10g P.S.B. + 10 g N.F.B+ O.W.	1.82	1.14	0.83	0.56
T_3 = 10g P.S.B. + 10 g N.F.B+ O.W+ 10 E.W	1.77	1.13	0.86	0.72
T_4 = 10g P.S.B. + 10 g N.F.B+ O.W+ 20 E.W	1.83	1.19	1.046	0.34
T_5 = 10g P.S.B. + 10 g N.F.B+ O.W+ 30 E.W	1.82	0.99	0.94	0.55
T_6 = 10g P.S.B. + 10 g N.F.B+ O.W+ 40 E.W	1.97	1.12	0.91	0.62
T_7 = 10g P.S.B. + 10 g N.F.B+ O.W+ 50 E.W	1.97	1.03	1.03	0.58
C.D.	**0.57**	**0.321**	**0.318**	**0.326**

T_1 : Control (OW containing cowdung and straw at 1:1 ratio); **T_2 :**OW + 10g phosphate solubilising bacteria (PSB) + 10g nitrogen fixing bacteria (NFB); **T_3 :**OW + 10gPSB + 10gNFB + 10 nos. earthworms (EW); **T_4 :** OW + 10g PSB + 10 g NFB + 20 nos. EW; **T_5 :** OW + 10g PSB + 10 g NFB + 30 nos. EW; **T_6 :** OW + 10g PSB + 10 g NFB + 40 nos. EW; **T_7 :**OW + 10gPSB + 10 g NFB + 50 nos. EW

Table 3: CEC (C.mol.(P^+) kg^{-1}) in different treatments during the period of incubation

Treatments	CEC at 15 days intervals	CEC at 30days intervals	CEC at 45 days intervals	CEC at 60 days intervals
T_1 = O.W. (500g CD + 500gStraw).	71.24	72.30	76.44	80.85
T_2 = 10g P.S.B. + 10 g N.F.B+ O.W.	80.96	80.96	86.84	114.27
T_3 = 10g P.S.B. + 10 g N.F.B+ O.W+ 10 E.W	115.00	118.34	118.34	114.92
T_4 = 10g P.S.B. + 10 g N.F.B+ O.W+ 20 E.W	116.56	109.86	109.86	124.28
T_5 = 10g P.S.B. + 10 g N.F.B+ O.W+ 30 E.W	94.76	113.66	113.66	133.06
T_6 = 10g P.S.B. + 10 g N.F.B+ O.W+ 40 E.W	113.28	133.59	133.59	136.28
T_7 = 10g P.S.B. + 10 g N.F.B+ O.W+ 50 E.W	116.26	132.57	132.57	143.78
C.D.	**20.61**	**22.40**	**23.88**	**29.49**

T_1 : Control (OW containing cowdung and straw at 1:1 ratio); **T_2 :**OW + 10g phosphate solubilising bacteria (PSB) + 10g nitrogen fixing bacteria (NFB); **T_3 :**OW + 10gPSB + 10gNFB + 10 nos. earthworms (EW); **T_4 :** OW + 10g PSB + 10 g NFB + 20 nos. EW; **T_5 :** OW + 10g PSB + 10 g NFB + 30 nos. EW; **T_6 :** OW + 10g PSB + 10 g NFB + 40 nos. EW; **T_7 :**OW + 10gPSB + 10 g NFB + 50 nos. EW

Table 4: Easily oxidizable and total organic carbon values (%) in the substrates after 60 days of incubation

Sample no.	Treatments.	O.C.%	Total O.C.%
1.	T_1 = O.W. (500g CD + 500gStraw).	7.80	26.32
2.	T_2 = 10g P.S.B. + 10 g N.F.B+ O.W.	7.08	23.12
3.	T_3 = 10g P.S.B. + 10 g N.F.B+ O.W+ 10 E.W	6.00	20.32
4.	T_4 = 10g P.S.B. + 10 g N.F.B+ O.W+ 20 E.W	6.12	19.64
5.	T_5 = 10g P.S.B. + 10 g N.F.B+ O.W+ 30 E.W	7.56	18.28
6.	T_6 = 10g P.S.B. + 10 g N.F.B+ O.W+ 40 E.W	8.04	18.96
7.	T_7 = 10g P.S.B. + 10 g N.F.B+ O.W+ 50 E.W	8.16	18.64

T_1 : Control (OW containing cowdung and straw at 1:1 ratio); **T_2 :**OW + 10g phosphate solubilising bacteria (PSB) + 10g nitrogen fixing bacteria (NFB); **T_3 :**OW + 10gPSB + 10gNFB + 10 nos. earthworms (EW); **T_4 :** OW + 10g PSB + 10 g NFB + 20 nos. EW; **T_5 :** OW + 10g PSB + 10 g NFB + 30 nos. EW; **T_6 :** OW + 10g PSB + 10 g NFB + 40 nos. EW; **T_7 :**OW + 10gPSB + 10 g NFB + 50 nos. EW