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Vermicomposting of Municipal Solid Waste Employing *Eisenia fetida* together with *Penicillium* spp. and *Azotobacter* Bioinoculants

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ABSTRACT

In the present study, the use of bioinoculants (*Azotobacter chroococcum*, *Penicillium chrysogenum* and *P. funiculosum*) in vermicomposting using an epigeic earthworm, *Eisenia fetida*, to transform the organic fraction of municipal solid waste (MSW) into valuable vermicompost, was explored. The organic fraction of MSW, when mixed with leaf litter (4:1), was treated with bioinoculants and processed for use as a vermicompost. Various biochemical parameters such as total organic carbon (TOC), total Kjeldhal nitrogen (TKN), EC, pH, available phosphorus (P), available potassium (K), cellulose, hemicelluloses and lignin were analyzed. The number of earthworms and the percentage nitrogen, P and K in vermicompost increased while pH and TOC declined as a function of the vermicomposting period. A 14-60% increase in TKN was observed in different microbial combinations at the end of the vermicomposting period. Available P increased 1.4- to 9.5-fold in different feed mixtures in comparison to the control. TOC was most reduced in the *E. fetida* treatment inoculated with a combination of *Azotobacter chroococcum* and *Penicillium chrysogenum* (37.2%) followed by *A. chroococcum* (36.1%) and *A. chroococcum* + *P. funiculosum* (33.8%). Analysis of three enzymes (β-glucosidase, phosphatase and urease) also showed better results in the *E. fetida* treatment together with all microbial combination than the control. β-glucosidase showed a significant increase (51%) while urease decreased sharply during the process. Vermicomposting using *E. fetida* + bioinoculants (*A. chroococcum* + *P. chrysogenum*) is a suitable technology for the decomposition of different types of organic wastes (domestic and industrial) into value-added material.

Keywords: compost, efficient microbes, epigeic earthworm, municipal solid waste, organic waste, rapid composting, solid waste management
Abbreviations: CMSW, compostable municipal solid waste; **TOC**, total organic carbon; **TKN**, total Kjeldhal nitrogen

INTRODUCTION

Urban populations are increasing worldwide with a concomitant increase in waste. In India, there are few landfill sites and the most common method of disposing such wastes is to haphazardously dump them in low-lying areas on the outskirts of towns. This has serious environmental impacts: Soil and water pollution, emission of methane, communicable diseases and creation of obnoxious and unhygienic conditions (Rao and Shantaram 1996). Biological contaminants are also found in municipal solid waste (MSW) and pathogens are likely to come from dirty discarded cloth, faeces of domestic animals, sanitary tissue papers or putrifying food (Pehren and Clark 1987). In addition, the use of chemical fertilizers and pesticides in agriculture deplete soils of indigenous nutrients and organic matter, and result in wide-scale surface and groundwater contamination (DeLuca and DeLuca 1997; Fauziah and Agamuthu 2009). Nonetheless, the loss of soil organic matter due to intensive agricultural practices is responsible for a decrease in soil fertility (Rasmunsen and Collins 1991; Adi and Noor 2009). The most common practice to preserve and/or restore soil fertility is to add organic matter (Costa *et al.* 1991), which preferentially, should be sufficiently stabilized – biochemically – to produce beneficial effects (Gallardo and Nogales 1987; Mathur *et al.* 1993). In India, MSW is composed of about 43.95% vegetative matter (paper, fruits and vegetables waste, food waste, etc.) and is generally termed compostable MSW (CPCB 2000). Thus, to obtain sufficient organic soil amendments, considerable research efforts have been made in recent years to investigate and develop various processes of organic matter stabilization and management.

Vermicomposting is well known for stabilizing different natural and anthropogenic wastes (Kale *et al.* 1982; Elvira *et al.* 1996; Edwards 1998; Singh and Sharma 2002). It is an aerobic, bio-oxidative, stabilizing, non-thermophilic process of organic waste decomposition that employs earthworms to fragment, mix and promote microbial activity (Gaundi *et al.* 2003). Although microbes are responsible for the biochemical degradation of organic matter, earthworms are the important drivers of the process, conditioning the substrate and altering its biological activity. The earthworm gut, which serves as a bioreactor, provides a suitable environment for the multiplication of microbes. Earthworms transform energy-rich and complex organic substances into a stabilized humus-like product called vermicompost which contains most nutrients in plant-available forms as well as plant growth-promoting substances like cytokinins and auxins (Krishnamoorthy and Vajrabhiah 1986) and consistently promote biological activity for germination, plant growth, flowering and better yield. However, there is an inherent risk in the use of unhygienic compost prepared from the organic fraction of MSW (Engeli *et al.* 1993; Arancon *et al.* 2005).

Lignin is the most recalcitrant material present in organic wastes and hardest to degrade in the composting process (Manna *et al.* 2003). However, some higher fungi have

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the ability to degrade lignin; *Phanerochaete chrysosporium* is considered to be the most effective lignin-decomposing white-rot fungus (Chahal 1994). Moreover, earthworms necessarily have to feed on microbes, particularly fungi, for their protein/nitrogen (N) requirements in reproduction and growth (Parthasarthi and Ranganathan 1999). *Trichoderma viridae*, which produces cellulase and has copper-bioaccumulating ability (Anand *et al*. 2006), is also considered to be a consistently effective inoculant for composting (Bhardwaj and Gaur 1985). Singh and Sharma (2002, 2003) reported that the inoculation of N-fixing bacteria during composting increased the N content of a stabilized product but the N-fixing ability of bacteria depended on the organic waste used (Beauchamp *et al.* 2006). Some fungi may be able to solubilise phosphate and can improve the phosphorous (P) nutrition of plants and thus stimulate plant growth. *Penicillium radicum*, a phosphate-solubilising fungus has shown promise in promoting plant growth (Whitelaw *et al.* 1999). All the microbes present in organic wastes are not killed during passage through the earthworm gut (Hendrikson 1990); in fact, the microbial population increases in the ejected material (Fischer *et al.* 1997). Therefore, microorganisms could be used as inoculants in vermicomposting. The total amount of bacteria in linings and earthworm casts is higher than in surrounding bulk soil (Edwards and Fletcher 1988; Fisher *et al.* 1995). Vermiculture beds with the bacterium *Acinetobacter calcoaceticus* stimulated earthworm growth and the consumption of cow slurry (Hand *et al.* 1988).

Hence, the quality of a vermicompost depends on various factors, but mainly on the nature of the substrate (organic residue), the earthworm species used and the role and efficiency of dominating microorganisms in the process (Singh and Sharma 2003; Sharma *et al.* 2005). Therefore, it is necessary to evaluate the efficacy of biocontrol agents like *Penicillium* sp. not only in accelerating the decomposition process but also in obtaining pathogen-free quality compost. The quality of the final product (i.e., vermicompost) can be ascertained by assessing its enzymatic activity and specific chemical and microbial characteristics such as total organic carbon (TOC), total Kjeldahl nitrogen (TKN), C:N ratio, P and potassium (K) content, cellulose, hemicelluloses and lignin content, humic acid content, etc. It would also help by understanding the dynamics of vermicomposting. Biochemical parameters, especially enzymatic activity, are indicators of the advancement of the composting process (Benitez *et al*. 2005; Pramanik and Chung 2010). However, literature on their evaluation during vermicomposting is still scanty. The application of vermicompost enhances the microbial and enzymatic activity of soils (Aira *et al.* 2007). Syers and Springett (1984) found that an increase in the number of bacteria and actinomycetes resulted in enhanced phosphatase activity in earthworm (*Lumbricidae*) casts. Extracellular enzyme activity may increase during vermicomposting of wastes due to the continuous accumulation of cell-released (extracellular) enzymes in humic matter, which become stabilized and resistant to physical and microbial degradation (Benitez *et al.* 2000; Singh and Sharma 2003; Sharma *et al*. 2005).

Thus, the aim of this study was to investigate the effect of bioinoculants on earthworms and subsequently on the quality of vermicompost and on enzymatic activities during the vermicomposting of compostable MSW.

MATERIALS AND METHODS

Experimental layout

The MSW from the residential and hostel areas of IIT Delhi was first collected at a waste collection site and then segregated manually into recyclable and organic fraction/compostable fractions. In this study, only the compostable fraction was used in experiments. The organic fraction of MSW, together with a mixture of leaf litter (4: 1), was selected as a substrate. The leaf litter included the dry leaves of *Leucaena leucocephala* and *Morus alba. Penicillium funiculosum* and *P. chrysogenum* were cultured and then subcultured on potato dextrose agar (PDA) at 28°C for 7 days then transferred to potato dextrose broth and grown at the same conditions. *Azotobacter chroococcum* was cultured on Jensson's agar at 28°C for 24 h then transferred to Jensson's broth and incubated. 7-days-old *P. funiculosum*, *P. chrysogenum* and 24-h-old *A. chroococcum* were inoculated in each treatment combination using 50 mL of each broth culture/kg of organic wastes. These pot bioreactors were maintained in this state for 10 days. Each treatment was repeated three times.

The most widely used earthworms for vermicomposting in India, *Eisenia fetida*, were obtained from an earthworm culture pit in Micromodel, IIT Delhi, where they had been cultured for the last 5 years. Initially, the species was collected from the culture bank of Gandhi Krishi Vignana Kendra, Bangalore.

The experiments were conducted in bioreactors (plant pots), each with a capacity of 1 kg of waste with a small hole at the bottom. One kg of organic MSW was placed in each pot and 10 healthy earthworms of the same size, each weighing 0.6-0.7 g, were introduced to the vermicomposting substrates. The treatments tested were: T1: Ef (*Eisenia fetida*); T2: Ef+Pf (*E. fetida* + *Penicillium funiculosum*); T3: Ef+Pc (*E. fetida* + *Penicillium chrysogenum*); T4: EF+Ac (*E. fetida* + *Azotobacter chroococcum*); T5: Ef+Ac+Pf (*E. fetida* + *A. chroococcum* + *P. funiculosum*); T6: Ef+Ac+Pc (*E. fetida* + *A. chroococcum* + *P. chrysogenum*).

The moisture content of the substrates was maintained between 70 and 80% throughout the vermicomposting period and the pots were maintained in darkness at room temperature. The ambient temperature recorded was between 28 and 35°C during the experimental period. During the vermicomposting period (60 days), the number of earthworms was counted every 20 days. The substrate in the bioreactors was removed, earthworms were selected by hand, counted and the mean of three replicates was used to express the results. After properly mixing of the content in the treatments, a sample of organic substrates was removed for analysis every 20 days. Then, all earthworms and substrates were returned to the bioreactor. Substrate samples collected during the vermicomposting period and at the end of the experimental period were stored in plastic vials at 40°C until chemical and biochemical analyses.

Chemical analysis

The chemical analysis of substrates and vermicompost samples, collected after every 20 days, was performed: TOC (Walkey and Black 1934); TKN (Singh and Pradhan 1981); cellulose, hemicellulose and lignin content (Dutta 1981). EC and pH were analysed by an EC and pH meter (2004 model, Scientific Systems), respectively. Available K was estimated by using ammonium acetate in a flame photometer (128, Systronics) while available P was determined using ammonium molybdate with a spectrophotometer (UV–160 A, Shimadzu) (Bray and Kurtz 1945).

Biochemical analysis

The activity of hydrolases (β-glucosidase, phosphatase and urease) was determined using a spectrophotometer (UV–160 A, Shimadzu) as described by Thimmaiah (2004). β- glucosidase (EC 3.2.1.21) activity was assessed by measuring the amount of aglocone. Folin-Ciocalteu reagent specifically reacts with the phenol moiety and in the presence of sodium carbonate, forms a chromatic complex which was measured by a spectrophotometer at 650 nm. Urease (EC 3.5.1.5) activity was determined by measuring the amount of NH_3 formed, which was measured by a spectrophotometer at 630 nm after the formation of indophenol blue. Phosphatase (EC 3.1.3.1) activity was measured by using the substrate *p*-nitrophenol phosphatase and by measuring the amount of *p*-nitrophenol formed by hydrolytic activity of the enzyme spectrophotometrically at 450 nm and pH 8.

Statistical analysis

All the reported data are the means of three replicates with standard deviations. Significant differences among the means of dif-

ferent bioinoculants inoculated for vermicomposting were estimated using Duncan's multiple range test (DMRT) at $P < 0.05$ using SPSS ver 16.0.

RESULTS AND DISCUSSION

In view of the results obtained from previously lab-level trials and culture experiments related to the compatibility of efficient microbes with earthworms, these studies on the vermicomposting of compostable MSW were initiated. Various parameters to assess vermicomposting efficiency were analyzed.

Physiochemical characteristics of the substrate used for vermicomposting

The substrate, comprising of an organic fraction of MSW and leaf litter in a 4:1 ratio, was prepared and analyzed for its physico-chemical properties before the earthworms and bioinculants were introduced into it. The percentage of TOC, TKN, P and K was found to be 31.3, 1.23, 0.21 and 0.32%, respectively. The organic waste was highly lignocellulosic because of the higher percentage of cellulose (33.1%), hemicellulose (28.4%) and lignin (18.4%). The waste was slightly acidic (pH 6.1) in nature with a suitable moisture content (65.3%) (**Table 1**).

Table 1 Characterization of the substrate used for vermicomposting.

Parameters	Values (%)
TOC	31.3 ± 2.1
TKN	1.23 ± 0.12
P	0.21 ± 0.02
K	0.32 ± 0.04
Cellulose	33.1 ± 3.2
Hemicellulose	28.4 ± 1.3
Lignin	18.4 ± 1.5
pH	6.1 ± 0.4
EC	1.23 ± 0
Moisture	65.3 ± 2.0

All values are in percentage except EC (ms/cm) and pH

Earthworm development

The effect of bioinoculants on growth of *E. fetida* during 60 days' vermicomposting of compostable MSW is shown in **Table 2**. In some cases mortality of earthworms was observed after 20 days. The non-homogenous nature and presence of some toxic metals in MSW (Garcia *et al.* 1993a) might not have supported earthworm growth in the substrates initially and caused mortality. The maximum number of *E. fetida* (59.3) was observed in the substrates inoculated with *A. chroococcum.* The presence of *A. chroococcum* in these substrates might be a reason for their survival. These results are supported by initial studies by Kaviraj and Sharma (2003) which suggested that a higher number of earthworms when inoculated with *A. chroococcum* in the substrate. There is evidence that some earthworms utilize microorganisms in their gut as a source of food (Edwards and Bohlen 1996; Edwards 1998) and that microorganisms provide a source of nutrients for earthworms with fungi as a major source and bacteria as a minor source (Edwards and Fletcher 1988). Earthworms necessarily have to feed on microbes, particularly fungi, for their protein/nitrogen requirement and for their reproduction and growth (Parthasarathi and Ranganathan 2000; Pattnaik and Reddy 2009).

Table 2 Effect of various bioinoculants on number of earthworms (juveniles) in municipal solid waste as substrate.

Bioinoculant	Number of earthworms			
	Day 0 (earthworms introduced)	20th day	40th day	60th day
Ef	10	9.3 ± 0.4 b	23.1 ± 3.6 b	51.3 ± 8.4 b
Ef+Pf	10	9.6 ± 0.2 b	22.3 ± 4.2 b	52.6 ± 5.4 b
Ef+Pc	10	9.0 ± 0.6 b	23.3 ± 1.4 b	53.3 ± 4.6 b
Ef+Ac	10	10.0 ± 2.2 a	26.3 ± 2.4 a	59.3 ± 3.4 a
Ef+Ac+Pf	10	9.6 ± 0.4 b	24.4 ± 4.1 b	58.0 ± 6.2 a
Ef+Ac+Pc	10	9.6 ± 0.6 b	28.2 ± 3.1 a	58.0 ± 2.2 a

Ef – *E. fetida*; Pf – *P. funiculosum*; Pc – *P. chrysogenum*; Ac – *A. chroococcum*.
All values are mean ± SD of three replicates; Different letters within a column denote significant differences at $P < 0.05$ according to DMRT.

Table 3 Effect of bioinoculants on percentage of TOC in vermicompost.

Bioinoculant	Total Organic Carbon (%)			
	Day 0 (earthworms introduced)	20th day	40th day	60th day
Ef	10	28.7 ± 1.3 b	24.1 ± 1.1 b	20.0 ± 2.2 b
Ef+Pf	10	27.2 ± 0.2 a	24.4 ± 1.7 b	20.7 ± 3.2 c
Ef+Pc	10	27.4 ± 3.5 a	24.8 ± 0.5 b	18.2 ± 0.8 a
Ef+Ac	10	27.1 ± 1.2 a	22.2 ± 3.7 a	17.3 ± 1.2 a
Ef+Ac+Pf	10	26.6 ± 4.1 a	22.4 ± 2.7 a	17.6 ± 2.6 a
Ef+Ac+Pc	10	26.6 ± 3.1 a	23.2 ± 1.4 b	16.7 ± 2.1 a

Ef – *E. fetida*; Pf – *P. funiculosum*; Pc – *P. chrysogenum*; Ac – *A. chroococcum*.
All values are mean ± SD of three replicates; Different letters within a column denote significant differences at $P < 0.05$ according to DMRT.

Total organic carbon

The data pertaining to the role of different combinations of bioinoculants in vermicomposting on reduction of TOC in substrates is provided in **Table 3**. The loss in TOC in all substrates using bioinoculants was significantly higher ($P < 0.05$) than the control (without bioinoculants). Maximum reduction in the percentage of TOC (16.7%) was observed in the substrate inoculated with *A. chroococcum* and *P. chrysogenum* along with *E. fetida*, i.e., T6. Here, a higher rate of mineralization might have been related to the higher number of earthworms in these substrates since earthworms are reported to increase the rate of mineralization of organic substrates (Singh and Sharma 2002; Kaviraj and Sharma 2003). When organic waste passes through the intestine of an earthworm, suitable conditions for a microorganism's growth results in microbial degradation of organic matter (Munnoli *et al.* 2000; Suthar 2009).

Many researchers have already reported the loss of organic matter (TOC) in terms of CO_2 in the process of aerobic fermentation and respiratory activity of earthworms and microorganisms (Senapati *et al.* 1980; Elvira *et al.* 1998). Our results corroborate those of Vinceslas and Loquet (1997), who reported a loss in TOC from 26 to 45% during vermicomposting. In the present study, the loss in TOC was approximately 45% in the final vemicompost.

Total Kjeldahl nitrogen

The loss of dry mass (organic carbon) in terms of CO_2 as well as water loss by evaporation during mineralization of organic matter (Viel *et al.* 1987) might have determined the relative increase in the percentage of TKN in the substrate during vermicomposting (**Fig. 1A**). The maximum increase of N (3.35%) was observed in the substrate inoculated with *A. chroococcum* and *P. chrysogenum* along with *E. fetida* (T6). The inoculation of *Azotobacter* in these combinations might have been a reason for the significant ($P < 0.05$) enhancement in percentage of N over other treatments (without bioinoculant). The inoculation of individual bioinculants was less effective than the combinations in terms of the percentage decrease of TOC and increase in NPK respective to the treatment with earthworms (i.e., T2).

Many studies have reported accelerated decomposition and improved N content in compost due to inoculation of *Azotobacter* (Singh and Sharma 2002, 2003; Edwards 2004; Saha *et al*. 2008). However, in general, the final N content in vermicompost is dependent on initial N present in the waste and the extent of decomposition (Crawford 1983; Gaur and Singh 1995; Suthar and Singh 2008) but also on the addition of N in the form of mucus, nitrogenous excre-

tory substances, growth-stimulating hormones and enzymes from earthworms (Tripathi and Bhardwaj 2004). These N-rich biochemical substances were not already present in the substrate hence they may also have acted as additional amendment to enhance the N level. Senapati *et al.* (1980) also reported an enhanced level of N in a closed vermicomposting system.

Available phosphorus and potassium

The combination of *A. chroococcum* and *P. chrysogenum* (T6) when inoculated into substrate increased the percentage of P as a consequence of the loss in TOC in the corresponding substrate (**Fig. 1B**). The inoculation of phosphate-solubilizing fungi *P. funiculosum* and *P. chrysogenum* into these substrates might have acted on the organic P and solubilized it into plant-available forms which might have led to enhanced P content.

The existence of soil microorganisms capable of transforming soil P to forms available to the plant has been recorded by many investigators (Kucey *et al.* 1989; Lazcano *et al.* 2008). Furthermore, inoculation of N-fixing bacteria (*A. chroococcum*), besides fixing N, could have possibly solubilized P due to production of organic acids and enzymes (Kumar and Narula 1999; Saha *et al*. 2008) and might have contributed to enhancing the P percentage. Laboratory studies, reviewed by Stevenson (1967), Kucey *et al.* (1989) and Bar-Yosef (1991), have shown that the solubilisation of soil phosphates is due to the excretion of organic acids by certain fungi. Fungi secrete organic acids and hence are reported to solubilise phosphate either by decreasing the pH or by complexing the cation which is bound to P. Moreover, there is also a rise in the P content during vermicomposting because of mineralization and mobilization of P which is due to bacterial and faecal phosphatase activity of earthworms (Krishnamoorthy 1990; Bijaya *et al.* 2007). Herein inoculation of *A. chroococcum* into these substrates increased the number of earthworms and subsequently increased the rate of mineralization and also contributed to a higher percentage of P.

A remarkable increase in P (51%) and K (38%) content in *P. excavatus*-worked vermicompost of sugarcane trash and cow dung substrate, compared to the control, was reported by Ramalingam and Thilagar (2000). The microflora influenced the level of available K and acid production by the micro-organisms, and is the major mechanism for solubilizing insoluble K during vermicomposting (**Fig. 1C**). A maximum increase in exchangeable K (1.92%) was achieved in the substrate inoculated with *A. chroococcum* with *P. chrysogenum* along with *E. fetida* (T6). The enhanced number of microflora present in the gut of earthworms might have played an important role in the mineralization of K and increased K_2O more than the control (Parthasarathi and Ranganathan 2000a).

Effect of bioinoculants on the enzymatic activity during vermicomposting

1. β-glucosidase activity

β-glucosidase activity changed throughout the vermicomposting period (**Table 4**). After 40 days, the enzymatic activity in the substrates inoculated with bioinoculant combination (*A. chroococcum* and *P. chrysogenum* (T6), *A. chroococcum* and *P. funiculosum* (T5), and *A. chroococcum* alone (T4)), showed almost complete stabilization of the substrates because much less enzymatic activity in these substrates was noted during the latter days i.e. after 60 days of experiment.

Increasing enzyme activity reflects rapid mineralization by high microbial metabolism. The presence of a high percentage of degradable organic compounds, available in the organic fraction of MSW, might have stimulated enzyme synthesis (Ceccanti and Garcia 1994; Pramanik *et al*. 2007; Pramanik and Chung 2010). β-glucosidase, which is a hydrolytic enzyme involved in the C cycle, showed a sharp increase during first 40 days in all substrates inoculated with bioinoculants. However, the enzymatic activity, after 40 days, was highest in the substrate inoculated with *A. chroococcum* and *P. chrysogenum* along with *E. fetida* (T6) (213.6 µmol PNP/g/h). This suggests that β-glucosidase is synthesised and released during vermicomposting. This is probably related to an increase in β-glucosidase-inducing

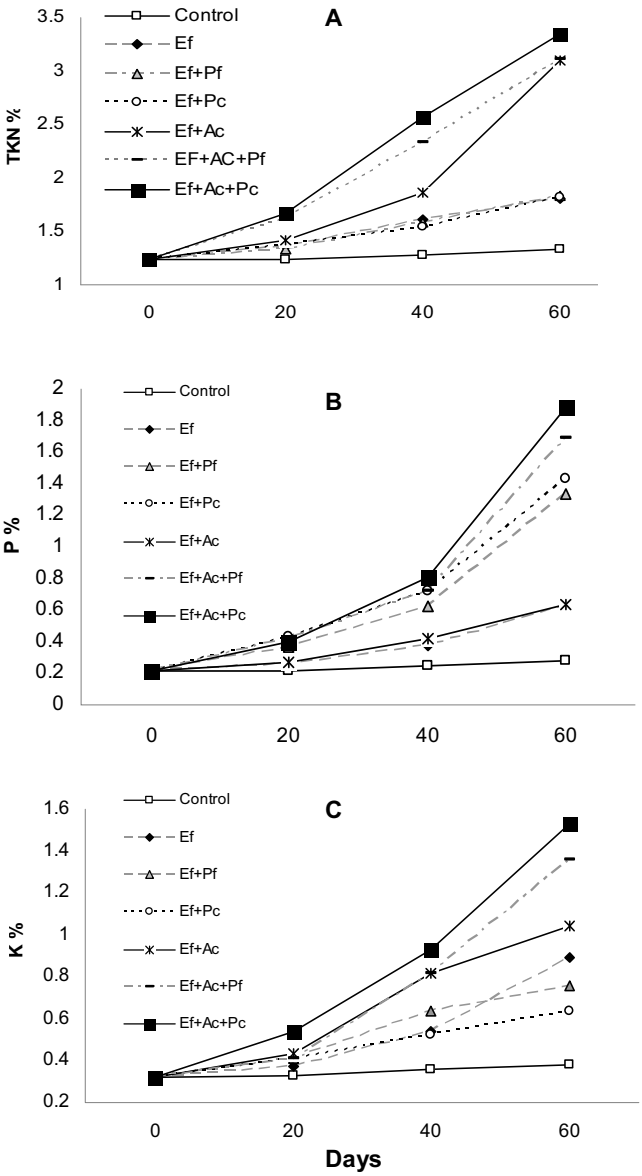

Fig. 1 Effect of bioinoculants on percentage of TKN (**A**), P (**B**) and K (**C**) in vermicompost. Ef – *E. fetida*; Pf – *P. funiculosum*; Pc – *P. chrysogenum*; Ac – *A. chroococcum*. All values are mean ± SD of three replicates.

Table 4 Effect of bioinoculants on β-glucosidase activity during vermicomposting.

Bioinoculant	β-glucosidase µmol PNP $g^{-1}h^{-1}$ (135.6)*			
	Day 0 (earthworms introduced)	20th day	40th day	60th day
Ef	10	145.4 ± 5.7 b	150.5 ± 7.8 a	149.1 ± 6.7 a
Ef+Pf	10	150.6 ± 9.4 b	185.6 ± 3.8 b	184.0 ± 3.8 b
Ef+Pc	10	151.2 ± 5.8 b	191.6 ± 6.6 b	190.3 ± 3.2 b
Ef+Ac	10	162.5 ± 8.9 c	194.5 ± 3.5 b	192.8 ± 2.3 b
Ef+Ac+Pf	10	171.3 ± 8.0 d	205.3 ± 8.9 c	201.5 ± 1.6 c
Ef+Ac+Pc	10	170.4 ± 8.5 d	215.4 ± 6.5 d	212.9 ± 2.7 d

Ef – *E. fetida*; Pf – *P. funiculosum*; Pc – *P. chrysogenum*; Ac – *A. chroococcum*.
All values are mean ± SD of three replicates; Different letters within a column denote significant differences at $P < 0.05$ according to DMRT.

Table 5 Effect of bioinoculants on phosphatase activity during vermicomposting.

Bioinoculant	Phosphatase µmol PNP $g^{-1}h^{-1}$ (334.4)*			
	Day 0 (earthworm introduced)	20th day	40th day	60th day
Ef	10	355.5 ± 7.8 ab	370.3 ± 14.5 b	356.3 ± 12.3 ab
Ef+Pf	10	357.6 ± 6.7 ab	372.5 ± 14.5 b	359.3 ± 8.9 ab
Ef+Pc	10	355.2 ± 9.4 b	371.0 ± 12.9 b	356.6 ± 6.7 b
Ef+Ac	10	360.2 ± 4.5 ac	380.4 ± 6.7 c	362.0 ± 8.3 c
Ef+Ac+Pf	10	367.5 ± 8.4 d	390.7 ± 12.4 d	367.2 ± 12.3 d
Ef+Ac+Pc	10	369.3 ± 9.4 d	389.9 ± 16.4 d	370.1 ± 6.2 d

Ef – *E. fetida*; Pf – *P. funiculosum*; Pc – *P. chrysogenum*; Ac – *A. chroococcum*.
All values are mean ± SD of three replicates; Different letters within a column denote significant differences at $P < 0.05$ according to DMRT.

Table 6 Effect of bioinoculants on urease activity during vermicomposting.

Bioinoculant	Urease µmol $NH_3 g^1h^1$ (478.8)*			
	Day 0 (earthworm introduced)	20th day	40th day	60th day
Ef	10	401.5 ± 21.3 c	154.5 ± 12.3 e	71.4 ± 6.7 d
Ef+Pf	10	386.4 ± 22.3 b	167.4 ± 6.7 e	68.6 ± 3.4 b
Ef+Pc	10	379.4 ± 24.5 b	162.3 ± 13.7 e	65.8 ± 5.4 b
Ef+Ac	10	323.1 ± 10.6 a	134.5 ± 5.9 d	64.5 ± 6.7 b
Ef+Ac+Pf	10	311.4 ± 13.4 a	102.3 ± 8.6 a	50.7 ± 4.9 a
Ef+Ac+Pc	10	306.9 ± 13.4 a	96.7 ± 3.9 a	53.1 ± 5.7 a

Ef – *E. fetida*; Pf – *P. funiculosum*; Pc – *P. chrysogenum*; Ac – *A. chroococcum*.
All values are mean ± SD of three replicates; Different letters within a column denote significant differences at $P < 0.05$ according to DMRT.

substrates released during the biodegradation of these wastes and/or an increase in microbial growth that produces an increase in enzyme synthesis (Eivazi and Tabatabai 1990; Pramanik *et al.* 2007; Lazcano *et al.* 2008).

β-glucosidase activity decreased slightly after 60 days, indicating that there was a decrease in microbial growth as a consequence of a decrease in organic matter (Garcia *et al.* 1993a, 1993b) and/or that microorganisms reduced enzyme synthesis due to decomposition of available substrates. However, the possibility that enzyme synthesis is repressed by particular metabolites or heavy metals, present in MSW, should not be ignored (Burns 1978).

2. Phosphatase activity

The inoculation of microorganisms into the substrates hastened the enzymatic activity of phosphatase and maximum activity (390.7 µmol PNP/g/h), observed with the inoculation of *A. chroococcum* and *P. funiculosum* along with *E. fetida* (T6) (**Table 5**). Phosphatases are enzymes with relatively broad specificity, capable of hydrolyzing various organic phosphate esters. The faster rise in phosphatase activity between 20 and 40 days, in two substrates i.e. compostable MSW with *A. chroococcum* with *P. chrysogenum* (T6) and compostable MSW with *A. chroococcum* and *P. funiculosum* (T5), might have been due to the increase of earthworm total biomass which stimulated microbial metabolism. Microbes, when entering earthworm guts, consume nitrogenous compounds of the mucus (Zhang *et al.* 2000), which largely increase their activity, which in turn enables them to contribute enzymes in the digestive processes of the earthworms.

Our results are corroborated by those of Benitez *et al.* (2005), who reported similar pattern of phosphatase activity during vermicomposting of sewage sludge employing *E. fetida*. Extracellular phosphatase activity increased until 40 days of vermicomposting, coinciding with the beginning of the depletion of earthworm biomass (Benitez *et al.* 2002). This suggests that earthworms and microorganisms had consumed the main P-cycle metabolizable substrates, indicating a relationship between the increase in microbial activity and the accumulation of phosphatase enzyme in the compost processing. After that, phosphatase activity decreased, showing similar values at the initial and final stages of vermicomposting.

3. Urease activity

The activity of urease, which catalyses the hydrolysis of urea to CO_2 and NH_4^+, decreased very rapidly until 40 days then remained more or less stable in the bioinoculated substrates (**Table 6**). After 60 days, minimum urease activity (50.7 µmol NH_3/g/h) was observed in the substrate inoculated with *A. chroococcum* and *P. funiculosum* (T5), followed by *A. chroococcum* and *P. chrysogenum* (T6) (53.1 µmol NH_3/g/h), which are significantly ($P < 0.05$) less than the control. Syers and Springett (1984) reported that urease activity was correlated to the initial TOC in the substrates. The fastest reduction was observed from the substrate inoculated by *A. chroococcum* with *P. chrysogenum* (T6) followed by *A. chroococcum* with *P. funiculosum* (T5). There was a sharp decrease in urease extracellular activity during vermicomposting, suggesting that N-substrates were consumed during the first few months of the degradation process (Benitez *et al.* 2005). The pattern was similar to that reported by Ceccanti and Garcia (1994) for a composting process in which proteinases transformed proteins into smaller N-compounds and ammonia (Nannipieri *et al.* 1990).

CONCLUSIONS

Vermicomposting can be a suitable alternative for environmentally safe stabilization of a final product (vermicompost), which could serve as excellent soil amendment in agriculture. MSW and a consortia of bioinoculants along with earthworms enhance the rate of biodegradation of the organic fraction of MSW mixed with leaf litter (4: 1) and improve the quality of vermicompost. The consortia of *E. fetida* + *A. chroococcum* + *P. chrysogenum* proved was the best combination of bioinoculants for making rapid and high quality vermicompost, in terms of available NPK, from the organic fraction of MSW. The enzymatic activity of β-glucosidase, phosphatase and urease in the vermicomposting substrate treated with bioinoculants suggested better quality and quantity compost than substrates without bioinoculants.

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