International Biodeterioration & Biodegradation 94 (2014) 90–97

Contents lists available at ScienceDirect

International Biodeterioration & Biodegradation

journal homepage: www.elsevier.com/locate/ibiod

Effect of fungal consortium and animal manure amendments on phosphorus fractions of paddy-straw compost

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ARTICLE INFO

Article history:
Received 13 May 2014
Received in revised form
24 June 2014
Accepted 24 June 2014
Available online 30 July 2014

Keywords:
Compost
Manures
Paddy straw
Phosphorus fractions

ABSTRACT

The study was conducted to reveal the type of phosphorus (P) fractions present in mature compost prepared by co-composting paddy straw (P.S) with cattle manure (CM), farm yard manure (FYM) and poultry manure (PM), each added separately as nitrogen (N) and P source. A consortium of phytate mineralizing fungi developed by including *Aspergillus niger* ITCC 6719, *Aspergillus flavus* ITCC 6720 and *Trichoderma harzianum* ITCC 6721 was applied for recovery of P from plant and animal residues. Chemical evaluation of compost after 4 months of aerobic decomposition revealed that inoculation improved the sodium bicarbonate-extractable P content of CM and FYM supplemented P.S compost by 32.3% and 23.5% respectively compared with their respective un-inoculated control. However, the peak values for water soluble-P fractions were recorded in CM–straw compost followed by PM–straw compost. Fungal inoculation also improved the agronomic quality of PM–straw compost as the latter had the highest total P content and lowest C:N and E4/E6 ratio of 18:1 and 5.36:1 respectively. The recovery of organic P from agricultural residue has the potential to reduce the application of synthetic P fertilizer. P-enriched organic manure can offer potential environment and economic benefits to farmers under sustainable agriculture.

1. Introduction

Animal manures have sufficient amount of nitrogen (N) and phosphorus (P), which are the main limiting nutrients in majority of agriculture soils. The direct land application of fresh animal manures results in excessive N and P input in soils and contamination of water bodies via run off (Li et al., 2012). High concentration of N and P in the soil profile is undesirable as excess manure adds salts in soils that decrease crop yields. The management of livestock manure for reduced loss of P and improved environment quality is important while recycling farm wastes as soil amendments. On the other hand, large proportion of rice crop residue produced annually (6.2 million tonnes) after the crop harvest (Pappu et al., 2011) is burnt in the open field itself. Burning causes loss of nutrients (C, N, P, and K) besides severely affecting the quality of ambient air and reduction of biological activity (Ghosh et al., 2004). For effective management of crop and animal residues, strategies such as composting are adopted by some countries. Composting converts labile organic compounds into stabilized product that can serve as an organic fertilizer.

Conventional decomposition of biodegradable wastes leads to production of compost with low P content ranging between 0.2 and 0.3%. Therefore, there is need to develop P-enriched compost that can partially substitute the input of chemical P fertilizer whose escalating cost is a financial constraint to marginal and small farmers. The mixing of high C:N ratio bulking agent such as paddy straw with low C:N ratio animal manure will bring down the C:N ratio of composting mixture to initiate the microbial decomposition. The organic P fraction present in paddy straw (84% of the total P) and animal manures (30–40% of total P) can be mineralized by phytate mineralizing microorganisms that can de-phosphorylate the organically bound phytate P and release it in the plant available inorganic (Pi) form. Recovery of organic P present in crop residue and livestock manure (60–65% feed P is excreted) can improve the P content of compost. Thus, co-composting of bioaugmented paddy straw and animal manure mixture may produce organic fertilizer with high P content. The progressive return of P-enriched organic fertilizer in soil can partially solve its problem of low P availability. Most of the studies for developing P-enriched compost have been confined to supplementation of composting substrates with rock phosphate and its subsequent inoculation with phosphate solubilizing microorganisms (Gaind and Gaur, 2000), but there is little or no information on use of phosphate

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http://dx.doi.org/10.1016/j.ibiod.2014.06.023
0964-8305/

mineralizing microorganisms for improving available P content of compost. The knowledge of total P content in compost or the increase in initial and final concentration of total P does not provide any information of the nature of the chemical forms of P present in compost. Thus, fractionation of compost-P can be an effective approach to know the availability, solubility and inter-conversion among P fractions (Shen et al., 2004). Hence, the objectives of the present investigation included (i) evaluation of phytate mineralizing fungi for improving the P content of compost (ii) estimation of P fractions in co-composted paddy straw and animal manure. The characterization of P in the farm waste compost will help to estimate the P supply capacity of the mature compost when used as soil amendment following land application.

2. Materials and methods

2.1. Phytate mineralizing fungi and inoculum development

Three potential phytate mineralizing fungi *Aspergillus niger* ITCC 6719, *Aspergillus flavus* ITCC 6720 and *Trichoderma harzianum* ITCC 6721 were isolated from different sources (Table 1) using phytate screening (PS) broth (Kerovuo et al., 1998). The pure cultures (obtained after repeated sub culturing) were maintained on potato dextrose agar (PDA) slants and stored in cold room.

The selected fungal isolates were evaluated for production of phosphatase, cellulase and phytase enzyme. Phosphatase activity was determined using *p*-nitrophenyl phosphate (pNPP) as substrate (Tabatabai and Bremner, 1969). The reaction mixture (1000 μl) consisting 250 μl of culture filtrate, 250 μl of 0.025 M pNPP and 500 μl of 0.1 M universal buffer (pH 6.5) were incubated at 37 °C for 1 h. The reaction was terminated by adding 2 ml of 0.5 M NaOH. Mixture was centrifuged at 4000*g* for 5 min. The intensity of yellow color developed was measured at 400 nm using UV–vis spectrophotometer. The amount of *p*-nitrophenol (pNP) released was quantified against pNP standard. One unit of phosphatase activity is the amount of enzyme required to release 1 μmol pNP h^{-1} ml^{-1} culture filtrate under the assay conditions.

Cellulase activity was analyzed by carboxymethyl cellulase (CMCase) and filter paper assay (FPase). A known sample (0.2 ml) of culture filtrate was incubated with 0.2 ml of carboxymethyl cellulose (CMC) and volume was made up to 1 ml with 0.05 M citrate buffer (pH 4.8) and incubated for 1 h at 50 °C in water bath. The amount of glucose liberated by the action of enzyme was estimated by adding 3 ml dinitro salicylic acid and keeping in boiling water bath for 16 min (Wood and Bhat, 1988). Enzyme concentration was represented as international unit (IU ml^{-1}). One IU is defined as μmol glucose produced min^{-1} ml^{-1} culture filtrate under the assay conditions. FPase activity was assayed using 50 mg dry Whatman No 1 filter paper as substrate instead of CMC.

For assay of phytase, 100 μl of culture filtrate was incubated with 100 μl phytate solution prepared in 0.4 M sodium acetate buffer of pH 5.6 containing 2 mM $CaCl_2$ (Engelen et al., 2001). After 1 h incubation at 37 °C, the reaction was stopped by adding 300 μl ammonium heptamolybdate/ammonium vanadate reagent solutions. All the assays were carried out in triplicate. The samples were centrifuged at 7000 rpm for 5 min. Supernatant was used for measuring the intensity of yellow color at 415 nm (Engelen et al., 2001) with UV–vis Thermo Fischer Scientific Spectrophotometer. One unit (U) of phytase activity was expressed as 1 μmol inorganic P (Pi) liberated min^{-1} under the assay conditions.

Inoculum of selected fungi was raised by immobilizing the respective strain on wheat bran. Flasks containing sterilized wheat bran moistened with mineral salt solution ($MgSO_4$, NH_4NO_3 and NaCl) were inoculated with 6 mm disc of each culture grown on PDA plates and incubated at 27 °C for 10 days. The whole growth of each strain including mycelium and spores was used as fungal inoculum.

2.2. Source material and composting experiment

Paddy straw (P.S) used as primary substrate and cattle manure (CM) and farm yard manure (FYM) used as N and P additives was obtained from Agronomy Division, Indian Agricultural Research Institute (IARI), New Delhi. Poultry manure (PM) was procured from one of the poultry farms located in peri-urban area of New Delhi, India.

Ten kg P.S, chopped to a size of 6–8 cm was filled in plastic bin of 80 l capacity and referred as T1. The dried CM, FYM and PM (each added separately) was thoroughly mixed with shredded P.S@15% (w/w) as indicated in Table 2

The initial moisture content of all the composting mixtures was maintained at 65–70% by adding water as per the requirement. The bins were covered with lids and mixture allowed to decompose. During the composting process, temperature of each bin was measured at different locations (top, middle and bottom) using thermometer. After 25 days of initial composting, when the temperature in the bins subsided to ~37 °C, the composting mixture in T6–T8 bins was inoculated with mixed fungal broth (obtained after mixing the growth of each strain separately with 200 ml sterilized water). T2–T4 served as their necessary checks. The composting mixture was aerated by manual turning at regular interval of two weeks. A moisture content of 60% was maintained during thermophilic phase and at 50% afterward. The composting period lasted for 4 months. The samples were drawn from different locations of the each bin. A total of ~1 kg sample was divided into two parts; one was preserved in the cold room for assay of microbial biomass carbon and phosphorus while the other was air dried, ground and passed through 2 mm sieve for subsequent chemical analysis.

2.3. Chemical analysis of compost

The pH and electrical conductivity (EC) measurements were performed on aqueous suspension of compost samples (1:5 w/v sample–water ratio) using digital pH meter and total dissolved salts (TDS) scanner respectively. The moisture content of different compost samples was determined on the basis of weight loss at 105 °C for 24 h. The organic matter (OM) content was estimated by weight loss on ignition at 550 °C for 5 h in a muffle furnace (Thermo Scientific Thermolyne bench top muffle furnace) as per protocol given by Tiquia et al. (2000).

$$\%\,OM = (W_{105} - W_{550}/W_{105}) \times 100 \quad (1)$$

Table 1
Enzyme profile of phytate mineralizing fungi.

Strain	Fungal Culture	Source	CMCase (IU ml^{-1})	FPase (IU ml^{-1})	Phytase (U ml^{-1})	APase (μmol pNP ml^{-1})
ITCC 6719	*Aspergillus niger*	Rhizosphere soil of *Glycine max*	145.22	6.91	103.3	367.73
ITCC 6720	*Aspergillus flavus*	Rhizosphere soil of *Pisum sativum*	82.22	9.45	85.43	830.07
ITCC 6721	*Trichoderma harzianum*	Compost	124.32	10.32	65.65	531.34

CMCase, carboxymethyl cellulase activity; FPase, filter paper activity; APase, acid phosphatase activity; IU, international unit; pNP, *p*-nitrophenol.