Ecological Engineering 116 (2018) 97–103

Contents lists available at ScienceDirect

Ecological Engineering

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

Quantifying the relative role of phytase and phosphatase enzymes in phosphorus mineralization during vermicomposting of fibrous tea factory waste

Swayambhu Ghosh[a,1], Anup Jyoti Goswami[a,1], Goutam Kumar Ghosh[b], Prabhat Pramanik[a,*]

[a] Soils Department, Tocklai Tea Research Institute, Tea Research Association, Jorhat 785008, Assam, India
[b] Department of Soil Science & Agricultural Chemistry, Palli Siksha Bhavana, Institute Of Agriculture, Visva-Bharati, Sriniketan 731236, Birbhum (Distt.), West Bengal, India

ARTICLE INFO

Keywords:
Tea factory waste
Cattle manure
Nutrients
Phosphorus mineralization
Phytase
Vermicomposting

ABSTRACT

Tea factory waste (TFW) is a difficult-to-degrade and fibrous waste material generated in tonnes during tea manufacturing. Though TFW contains high amount of nutrients; high phenolic compound content and fibrous nature limit its direct use in agriculture. In this study, TFW was mixed with cattle manure at different proportions for evaluating possibility of recycling TFW through vermicomposting. It was observed that earthworms (*Eudrilus euginae* used in this study) could only survive when TFW was mixed with cattle manure at least in 1:1 proportion (w/w basis). The quality of vermicompost prepared from 25% TFW and 75% cattle manure combination was comparable to the vermicompost of sole cattle manure. Phosphorus (P) is one of the major nutrients required for plant growth. Though the effect of phosphatase enzyme in P mineralization during vermicomposting is well established, phytase enzyme in the vermicomposted organic substrate was not quantified before. In this study, phytase enzyme, measured in vermicomposting materials, was positively related to the amount of P mineralized. Both acid phosphatase and phytase enzymes were responsible for P-mineralization during vermicomposting. It was observed that acid phosphatase enzyme contributed to the P-mineralization in the first 50 days, while phytase enzyme contributed in the latter part of vermicomposting.

1. Introduction

Vermicomposting is a mesophilic composting process that creates a conductive ambience for earthworms and microorganisms to mineralize complex substrates in a complementary manner (Pramanik and Chung, 2011). It is an easy, environment-friendly and cost-effective method for recycling organic wastes for producing nutrient-enriched soil amendment (Ali et al., 2012). Vermicomposting is the mineralization of organic substrates into plant-available ionic form (Pramanik, 2012). Phosphorus (P) is one of the major nutrients required for plant growth. Organic substrates possess P in the forms of monoesters, diesters and phytate and it is already established that phosphatase enzyme plays an important role in P mineralization during vermicomposting (Pramanik et al., 2009). However, phytate compounds contribute a major portion in organic P and can only be mineralized through phytase enzyme-mediated hydrolysis process to produce inorganic phosphate ions (Turner and Richardson, 2004). The presence of phytase enzyme and its influence in P mineralization during vermicomposting was not studied before.

Tea factory waste (TFW) is the remaining portion of plucked tea shoots after being processed for tea manufacturing. India is the second highest tea producerin world and contributes more 30% of total world production (Jibesh and Umesh, 2015). Each 100 kg plucked green shoot produce approximately 22 kg tea after being processed in the factory. However, about 82% of that tea is marketable for selling and remaining portion is discarded as waste. These TFW is generated in large amount and on an average weighs approximately 4% of the plucked green shoot (Wasewar et al., 2009). Annually, India alone produces 190,400 tonnes TFW. Those TFWs are mostly insoluble cell walls of tea leaves consisting largely of cellulose and hemicelluloses, lignin, condensed tannins and structural proteins (Uzun et al., 2010). Instead of its huge availability in tea-growing countries and high (> 2%) nitrogen (N) content; high phenolic content and fibrous nature limit the use of TFW as organic amendment in soil.

Vermicomposting is an effective technique to recycle various organic substrates into nutrient-rich soil amendment. Pramanik et al.

* Corresponding author.
E-mail address: prabhat2003@gmail.com (P. Pramanik).
[1] Authors contributed equally in this manuscript.

https://doi.org/10.1016/j.ecoleng.2018.03.001
Received 12 October 2017; Received in revised form 26 February 2018; Accepted 1 March 2018

(2010) found that mixing of cattle manure with organic wastes is required for providing initial energy source and favorable environment to the earthworms during vermicomposting. In this study, an attempt was made to recycle TFW through vermicomposting. To evaluate the optimum feeding composition, different proportions of TFW and cattle manure were fed to earthworms and different chemical and biochemical parameters were measured periodically throughout the vermicomposting period. Activities of phosphatase and phytase enzymes were compared with the concentration of inorganic phosphate ion during the process. The objectives of this study were to evaluate the suitability of vermicomposting for recycling TFW and to quantify the comparative influence of phosphatase and phytase enzymes in P mineralization during vermicomposting.

2. Materials and methods

2.1. Substrates used in the study

The experiment was conducted in the vermicomposting unit of Tocklai Tea Research Institute (TTRI), Jorhat, Assam, India. For this experiment, fresh cattle manure was collected from a nearby cowshed, while tea factory waste (TFW) was collected from the tea processing unit of the TTRI. The collected cattle manure was air-dried prior to using for vermicomposting experiment for removing ammonia gas from cattle manure. Initially, following five combinations of TFW and cattle manure were taken as treatments for this study – 100% TFW (T_1), 75% TFW and 25% cattle manure (T_2), 50% TFW and 50% cattle manure (T_3), 25% TFW and 75% cattle manure (T_4) and 100% cattle manure (T_5). In this study, 4 kg of all these combinations were taken in suitable size (10 L capacity) earthen pots for vermicomposting and all the treatments were arranged in triplicate for this study. Organic substrates in all the treatments were mixed properly, watered and allowed to partially decompose for 7 days. The purpose of this step was to make the organic substrates more palatable to earthworms. After 7 days degradation, 150 numbers of earthworms (*Eudrilus euginae*) were released in each tub for vermicomposting. The organic substrates at the time of earthworm introduction were considered as zero-day stage of this study. Chemical properties of raw organic wastes were presented in Table 1.

It was observed that earthworms in T_1 and T_2 treatments could not survive even after several trials during this study. Therefore, those two treatments were excluded from the ongoing experiment and three (3) treatments (T_3, T_4 and T_5) were finally vermicomposted in this study. Moisture contents in these treatments were maintained within 60–70% by periodical sprinkling of water throughout the study. Samples from all the treatments were collected at 15 days interval for analyzing total organic carbon (TOC), total Kjeldahl nitrogen (total N), total P, water-extractable P (WEP), total potassium (total K), humic acids, microbial biomass C (MBC), acid phosphatase activity (SPA) and phytase activity (PTA).

One portion of fresh samples was immediately analyzed for microbial and enzyme assays, while the other portion of the samples was dried for chemical analyses. The completion of vermicomposting was determined by constant values of chemical (TOC) and biochemical [SIR (calculated from MBC following the equation of Aira and Domínguez, 2010) ($\mu g\ CO_2\ h^{-1}\ g^{-1}$ dry VC) = 25.97 + 0.04 MBC ($\mu g\ g^{-1}$ dry VC)] parameters of organic substrates during vermicomposting.

Table 1
Chemical properties [#] of initial waste materials prior to vermicomposting.

Parameters	Raw materials	
	Tea factory waste	Cattle manure
Total organic carbon ($mg\ g^{-1}$)	556.0 ± 24.2	360.0 ± 15.9
Total Kjeldahl nitrogen ($mg\ g^{-1}$)	10.65 ± 0.05	4.60 ± 0.20
Carbon/nitrogen ratio	52.20	78.26
Total phosphorus ($mg\ g^{-1}$)	2.30 ± 0.02	3.60 ± 0.01
Total potassium ($mg\ g^{-1}$)	15.92 ± 0.10	3.40 ± 0.02

[#] All data represented mean ± standard deviation (n = 3) of the treatment for corresponding parameter.

2.2. Chemical analyses

Total organic C content was determined from dried samples following the method of Nelson and Sommers (1982). Total Kjeldahl N content in organic samples was determined by acid digested using concentrated H_2SO_4 followed by alkaline distillation (Bremner and Mulvaney, 1982). For total P and total K determination, 0.5 g dried sample was placed in muffle furnace for dry digestion (Kalra, 1997), and the ash was transferred quantitatively in 100 ml volumetric flask by dissolving in 0.01 N HCl solution. Aliquot of this solution was used for blue colour formation using ammonium molybdate and stannous chloride solutions and the colour intensity of the solution was recorded using spectrophotometer at 660 nm for total P measurement. The digested solution was directly used for total K was measurement by flame photometer. Water extractable P was measured by shaking the samples with 50 ml of double distilled water for 30 min. After filtering the solution, an aliquot was taken for spectrophotometric determination of WEP using ammonium molybdate and stannous chloride reagents.

2.3. Acid phosphatase and phytase activity

For SPA assay, fresh sample (0.5 g) was taken in a test tube and 0.25 ml toluene added to it for restricting microbial activity. The sample was then incubated with 4 ml modified universal buffer (pH 6.5) with 1 ml 50 mM p-nitrophenyl phosphate (Sigma: N4645) solution for one hour at 37 °C (Tabatabai and Bremner, 1969). After incubation,4ml of 0.5 M NaOH and 1 ml of 0.5 M $CaCl_2$ solutions were added to it to stop the reaction and the filtrate was used for measuring *p*-nitrophenol concentration at 420 nm against commercially available *p*-nitrophenol (Sigma: 241326) as standard.

For PTA estimation, 0.5 g of fresh sample was incubated with 5 ml citrate buffer (pH 5.5) and 1 ml of 0.2% sodium phytate (Sigma: P8810) solution as substrate for 1 h, adding 0.25 ml toluene to cease the microbial growth (Bae et al., 1999). After incubation, 3 ml of 0.5 M HCl and 1 ml of 0.5 M $CaCl_2$ were added to it. Phytase activity in the filtrate was determined spectrophotometrically by estimating phosphate ion concentration using ammonium molybdate and stannous chloride reagents. For this assay, KH_2PO_4 was used as standard. Data of both SPA and PTA were reported on the basis of dry weight of organic substrates.

2.4. Microbial biomass carbon

Microbial biomass carbon was determined by chloroform fumigation-extraction method (Joergensen, 1996). Fumigated and non-fumigated samples were extracted with 0.5 M K_2SO_4 solution, and C content was estimated by dichromate oxidation method (Vance et al., 1987). The MBC was calculated as MBC = (C in fumigated soil − C in non-fumigated soil)/K_c; where, K_c was the conversion factor (Sparling and West, 1988). The results of MBC were also reported on dry weight basis.

2.5. Humic acid quantification

Humic acids content in the final vermicompost samples was determined by classic alkali-acid fractionation procedure (Valdrighi et al., 1996). The vermicompost samples were digested with 0.1 N KOH solution for 24 h at room temperature. The organic residue was then separated from the liquid fraction by centrifugation at 5000 rpm for 30 min. The supernatant was acidified up to pH 2.0 with 6 N H_2SO_4solution and kept in dark for 24 h in order to flocculate humic acids. After acidification, precipitated humic acids were collected by