
ORIGINAL ARTICLE

Enzymes activity during Composting of Silkworm Pupal residue with Agricultural substrates

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ABSTRACT

An investigation was carried out on enzymes activity during composting of silkworm pupal residue with agricultural substrates. Significant variation in dehydrogenase, phosphatase and urease activity was noticed among the treatments at all the intervals during composting of silkworm pupal residue with other substrates. Dehydrogenase activity was found to be increased up to 30th day of composting and decreased upto 60th day thereafter, it gradually increased upto 120th day of composting. At the final stage of composting, the highest dehydrogenase activity was observed in the treatment T₃ (T₂+ silkworm litter) (198.10 µg TPF /g soil/hour) followed by the treatment T₇(T₂+vermicompost) (196.50 µg TPF /g soil/hour). The phosphatase and urease activity was found to be increased at all the intervals. The maximum acid and alkaline phosphatase activity was recorded in the treatment T₇ (T₂+vermicompost) (160.60 µg PNP g⁻¹hr⁻¹) and T₃ (T₂+ silkworm litter) (185.67 µg PNP g⁻¹hr⁻¹). At 120th day of composting, maximum urease activity was recorded in the treatment T₃ (T₂+ silkworm litter) (448.30µg NH₄⁺-N g⁻¹hr⁻¹) followed by the treatment T₇ (T₂+ vermicompost) (396.80 µg NH₄⁺-N g⁻¹hr⁻¹). The least dehydrogenase, urease, acid and alkaline phosphatase activity was in the treatment T₁ (silkworm pupal residue) (156.20 µg TPF g⁻¹ soil hr⁻¹, 340.00 µg NH₄⁺-N g⁻¹hr⁻¹, 68.10 and 63.60 µg PNP g⁻¹hr⁻¹ respectively).

Keywords: silkworm pupal residue compost, dehydrogenase, phosphatase, urease.

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INTRODUCTION

Silkworm litter and silkworm pupae are the two major by-products generated every year in substantial amount in silk industry as waste. The silkworm pupa is one of the major by-products of silk industry, which has been considered as waste in silk reeling unit [10]. Annually India produces about 40,000 MT of silkworm pupae on dry weight basis. The major difficulty in the utilization of spent silkworm pupae is, it cannot be stored for long period as it emits bad odour due to putrefaction [19]. Disposal of silkworm pupae meal was a big problem in the silk factories. Recently, the chemical compositions of silkworm pupae have attracted considerable attentions in the world and silkworm pupae are considered to be a good source of a large number of bioactive substances. The pupae contain about 79.8 per cent of protein, 6.6 per cent fat and 5.1 per cent ash. Dried silkworm pupae contain about 8 per cent nitrogen. The crude protein extracted with 0.5 per cent sodium hydroxide contains 12.22 per cent nitrogen [8]. Since the pupae contains high amount of nitrogen and protein along with micronutrients like zinc, copper, magnesium and manganese, there is a prospective potential for the bio-conversion of pupal waste to enriched compost and utilization as a nutrient source.

Composting is a biological decomposition of organic matter by microorganisms. During composting, the starting material is transformed through a variety of biological and biochemical processes in which enzymes play a role. [18]. The enzyme activities largely reflected the diversity of the microbial population and in turn reflect the composting process. The three enzymes viz., dehydrogenase, phosphatase and

urease were recognized as very important enzymes involved in the mineralization of nutrients. The mineralization of organic N during composting, which involves the release of N from non-peptide C-N bonds in amino acids and urea is mediated by enzymes such as amidohydrolases and dehydrogenases. Alkaline and acid phosphatases are important enzymes in organic P mineralization and plant nutrition [16].

Characterizing and quantifying enzymatic activities during composting can reflect the dynamics of the composting process in terms of the decomposition of organic matter and nitrogen transformations, and may provide information about the maturity of composted products [17]. In addition, on the basis of the well demonstrated relationship between enzymatic activity and quantity and quality of organic matter it could also give information on compost stability [6], defined as the degree of decomposition of the readily bio-degradable organic matter [7]. With this context the present study was conducted on "Enzymes activity during composting of silkworm pupal residue with agricultural wastes".

MATERIALS AND METHODS

Compost was prepared in cement cisterns of 100 cm X 50 cm (height X diameter). The silkworm pupal residue was mixed with other substrate in 2:1 ratio layer by layer, and microbial consortium (*Bacillus licheniformis*, *Aspergillus aculeatus*, *Penicillium simplicissimum* and *Streptomyces noursei*) was added to each layer at the rate of 2.5 kg per ton of substrate to improve the efficiency of composting as per treatment structure. Finally, the composting material was completely covered with soil slurry at the top in order to prevent the heat loss and emission of odour. Composting material was turned regularly once in 15 days to ensure aeration and water was regularly added to maintain the moisture with approximately 60 per cent of maximum water holding capacity.

The composting experiments were carried out in the Department of Agricultural Microbiology, UAS, GKVK, Bengaluru with following treatment combinations.

Treatment detail

T1: Silkworm pupal residue

T2: T1+ microbial consortium

T3: T2+ silkworm litter

T4: T2+ maize stover

T5: T2+ farm yard manure (FYM)

T6: T2+ urban solid waste

T7: T2+ vermicompost

The design adopted was completely randomized block design (CRD) with seven treatments and four replications.

Sample collection

During composting, samples were collected at different intervals (30, 60, 80 and 120 days) from each cistern (from four sides and one from center) using spiked augers. The samples were pooled in each treatment and composite samples were analyzed for enzyme activities by following the standard procedures.

Dehydrogenase activity

Dehydrogenase activity in the samples was determined by following the procedure described by Casida *et al.* (1964). Five grams of sample was thoroughly mixed with 0.2 g of CaCO₃ and transferred to test tubes. One ml of 1.5 per cent aqueous solution of 2,3,5-triphenyl tetrazolium chloride (TTC) was added to each tube. One ml of 1 per cent glucose solution and 8 ml of distilled water was added in such a way that it should form a thin film of water just above the sample layer. The tubes were stoppered and incubated at 30°C for 24 hours. At the end of incubation, the contents in the tube were rinsed down into a small beaker through Whatman No.15 filter paper. Repeated rinsing of sample with methanol was continued till the filtrate ran free of red color. The volume of the filtrate was made up to 50 ml with methanol in a volumetric flask. The intensity of red color was measured at 485 nm against a methanol blank using UV-VIS Spectrophotometer. The concentration of formazan formed in samples was determined by using graded concentrations of formazan. The results were expressed as microgram of triphenylformazan (TPF) formed per gram of sample per day.

Phosphatase activity

Phosphatase activity of samples was determined by following the standard procedure of Eivazi and Tabatabai [3]. One gram of sample was placed in 50 ml Erlenmeyer flask in 2 sets of which 0.2 ml toluene followed by 4 ml of modified universal buffer (pH 6.5 for acid phosphatase and pH 11.00 for alkaline phosphatase) was added to the flasks and contents of the flasks were mixed by swirling for 2 minutes. The flasks were stoppered and incubated at 37°C for 1 hour. After incubation, 1 ml of 0.5 M CaCl₂ and 4 ml of

0.5 M NaOH were added to the flask, swirled and filtered through Whatman No. 42 filter paper. The intensity of yellow color developed was measured at 420 nm against the reagent blank using UV-VIS spectrometer. Control was performed following the same procedure described above, except that the para-nitrophenyl phosphate solution was added after the addition of 0.5 M CaCl₂ and 0.5 M NaOH and just before filtration. The phosphatase activity in the samples was expressed as μg para-nitro phenol $\text{g}^{-1} \text{h}^{-1}$ of sample with reference to the standard curve prepared by using graded concentrations of para-nitrophenol solution.

Urease activity

The urease activity was estimated by following the method outlined by Eivazi and Tabatabai [3]. Two gram of sample was taken in a 50 ml Erlenmeyer flask and treated with 0.2 ml of toluene and 9 ml of THAM buffer and flasks were swirled for few seconds to mix the contents properly. One ml of 0.2 M urea was added and the flasks were swirled for few seconds and placed in an incubator at 37°C. The stoppers were removed after 2 hours and approximately 35 ml of KCl-Ag₂SO₄ solution was added. The flasks were swirled again and allowed to stand until the contents have cooled to the room temperature. The volume was then made up to 50 ml by adding KCl-Ag₂SO₄. The NH₄-N was estimated by steam distillation. Controls were performed in the same way, but 1 ml of 0.2 M urea was added after the addition of 35 ml of KCl-Ag₂SO₄ solution. The urease activity in the samples was expressed as μg NH₄-N/g soil/hour.

RESULTS AND DISCUSSION

Enzymes activity during composting of silkworm pupal residue with other agricultural substrates

Dehydrogenase activity

The dehydrogenase activity during composting of silkworm pupal residue with agricultural substrates showed significant variation among the treatments during all the intervals (Table 1). The activity of dehydrogenase enzyme was higher at 30th day of composting and then decreased up to 60th day of decomposition and there after it increased up to 120th day of composting in all the treatments at all the intervals. At the final stage of composting, the highest dehydrogenase enzyme activity was observed in the treatment T₃ (T₂+ silkworm litter) (198.10 μg TPF /g soil/hour) followed by the treatment T₇(T₂+vermicompost) (196.50 μg TPF /g soil/hour) and was significantly higher compared to all other treatments while the least enzyme activity was recorded in the treatment T₁ (silkworm pupal residue) alone (156.20 μg TPF $\text{g}^{-1} \text{soil hr}^{-1}$).

Dehydrogenase is an intracellular enzyme which is usually involved in microbial oxido-reductase metabolism and its activity was found to be highest in compost than in soil [5]. The dehydrogenase enzyme activity basically depends on the metabolic state of microbes and widely used to measure the metabolic activities, which in turn correlated with total microbial activity. In the present study, there was higher dehydrogenase activity in all the treatments during initial days and then decreased noticeably up to 60th day and thereafter again it increased up to 120th day. This could be due to presence of higher amount of available nutrients and organic carbon content during earlier period of decomposition. It is suggested that carbon is one of the factors stimulating activity of the enzyme [1, 20]. Similar kinds of results were also reported by Pedrazzini and McKee [11] and Saviozzi *et al.* [12].

Acid and alkaline phosphatase activity

The data on acid and alkaline phosphatase activity showed significant differences among the treatments at all the intervals during the process of composting of silkworm pupal residue with agricultural substrates (Table 2). There was an increase in both the enzymes activity throughout the period of degradation. The maximum acid and alkaline phosphatase activity was recorded on 120th day of decomposition and was in the treatment T₇ (T₂+vermicompost) (160.60 μg PNP $\text{g}^{-1}\text{hr}^{-1}$) and T₃ (T₂+ silkworm litter) (185.67 μg PNP $\text{g}^{-1}\text{hr}^{-1}$) and are significantly higher when compared to all other treatments whereas, the least enzyme activity was observed in T₁ (silkworm pupal residue) (68.10 and 63.60 μg PNP $\text{g}^{-1}\text{hr}^{-1}$ respectively).

Phosphatase plays an important role in transforming the organic phosphorus into the available form of phosphorus [9]. The phosphatase activity is due to the presence of phosphorylated compounds, which act as a substrate for the microorganisms to synthesize phosphatase and is considered as general microbial indicator [16]. In the present study both the enzymes activities in all the treatments were higher during thermophilic phase of decomposition. It is quite possible that addition of silkworm pupal residue which is rich in nutrients to a material containing high organic carbon resulted in rapid decomposition due to higher activity of heterotrophic microorganisms which were dominant and results in depletion of available phosphorus for the growth of microorganisms which induced them to liberate extracellular phosphatase for mineralization of organic phosphorus. The results obtained are in confirmation with the findings of Senthilraja [13] who also reported the enhanced phosphatase enzyme activities during the

composting of pressmud fibre. The increase in phosphatase activity might be attributed to higher temperature and higher bacterial population [2].

Urease activity

The results obtained on urease activity showed significant variations among different treatments at all the intervals during the process of composting (Table 3). There was an increase in urease activity throughout the period of composting. At 120th day of composting, maximum urease activity was recorded in the treatment T₃ (T₂+ silkworm litter) (448.30µg NH₄⁺-N g⁻¹hr⁻¹) followed by the treatment T₇ (T₂+ vermicompost) (396.80 µg NH₄⁺-N g⁻¹hr⁻¹) and which was significantly higher compared to all other treatments. However, the least urease activity observed in treatment T₁ (silkworm pupal residue) alone (340.00 µg NH₄⁺-N g⁻¹hr⁻¹).

The enzyme urease is responsible for breakdown of urea into CO₂ and NH₃. The higher urease activity may probably due to availability of easily degradable substances and higher content of nitrogen in the raw materials used, which would have stimulated microbial activity and, as a consequence, higher urease activity was found. Similar observations were also recorded by Solano *et al.* [15], Pallab De *et al.* [9], Frankken and Dick [4] and Skujins [14]. They reported that increased urease activity during composting process could be due to higher organic carbon and total nitrogen content which resulted in higher microbial activity.

Table 1: Changes in dehydrogenase activity during composting of silkworm pupal residue with agricultural substrates

Treatments	Dehydrogenase activity (µg TPF g ⁻¹ hr ⁻¹)			
	30DAC	60 DAC	90 DAC	120DAC
T ₁ :Silkworm pupal residue	135.6 ^c	111.4 ^g	147.9 ^f	156.2 ^g
T ₂ :T ₁ +microbial consortium	138.8 ^b	118.6 ^e	151.4 ^e	158.0 ^f
T ₃ :T ₂ +silkworm litter	141.7 ^a	143.4 ^b	169.0 ^a	198.1 ^a
T ₄ :T ₂ +maize stover	128.1 ^e	136.7 ^c	157.8 ^c	163.9 ^e
T ₅ :T ₂ +FYM	130.1 ^d	114.6 ^f	153.0 ^d	172.1 ^d
T ₆ :T ₂ +urban solid waste	129.0 ^d	133.2 ^d	149.0 ^f	173.7 ^c
T ₇ :T ₂ +vermicompost	137.6 ^b	148.4 ^a	161.8 ^b	196.5 ^b

Note: Mean values followed by the superscript in each column do not differ significantly at P=0.05 level by DMRT

DAC- Days after composting

Table 2: Changes in acid and alkaline phosphatase activity during composting of silkworm pupal residue with agricultural substrates

Treatments	Acid phosphatase (µg PNP g ⁻¹ hr ⁻¹)				Alkaline phosphatase (µg PNP g ⁻¹ hr ⁻¹)			
	30 DAC	60 DAC	90 DAC	120 DAC	30 DAC	60 DAC	90 DAC	120 DAC
T ₁	50.65 ^f	57.48 ^g	66.61 ^g	68.1 ^g	56.21 ^e	58.56 ^e	60.86 ^f	63.6 ^f
T ₂	56.31 ^e	63.18 ^f	71.43 ^f	76.8 ^e	69.21 ^d	70.14 ^d	74.73 ^e	74.11 ^e
T ₃	68.23 ^d	72.31 ^d	86.22 ^d	121.2 ^b	93.56 ^a	102.03 ^b	148.03 ^a	185.67 ^a
T ₄	75.31 ^c	83.57 ^c	89.29 ^c	75.32 ^f	79.79 ^b	92.15 ^c	99.21 ^d	102.4 ^d
T ₅	86.18 ^a	93.38 ^a	96.05 ^b	108.0 ^c	75.94 ^c	92.65 ^c	137.33 ^b	147.3 ^b
T ₆	51.56 ^f	67.83 ^e	76.21 ^e	79.5 ^d	93.43 ^a	108.52 ^a	132.6 ^c	139.6 ^c
T ₇	80.13 ^b	91.02 ^b	132.94 ^a	160.6 ^a	75.94 ^c	92.65 ^c	137.33 ^b	147.3 ^b

Note: Mean values followed by the superscript in each column do not differ significantly at P=0.05 level by DMRT

DAC- Days after composting

Table 3: Changes in the urease activity during composting of silkworm pupal residue with agricultural substrates

Treatment	Urease ($\mu\text{g NH}_4^+\text{-N g}^{-1}\text{hr}^{-1}$)			
	30 DAC	60 DAC	90 DAC	120 DAC
T ₁ :Silkworm pupal residue	225.0 ^f	284.9 ^e	305.3 ^g	340.0 ^g
T ₂ :T ₁ +microbial consortium	225.80 ^f	292.7 ^d	310.8 ^f	354.0 ^f
T ₃ :T ₂ +silkworm litter	235.3 ^c	321.4 ^b	339.4 ^a	448.3 ^a
T ₄ :T ₂ +maize stover	229.9 ^e	294.1 ^c	315.3 ^e	366.0 ^e
T ₅ :T ₂ +FYM	231.2 ^d	294.4 ^c	320.6 ^d	385.0 ^c
T ₆ :T ₂ +urban solid waste	237.4 ^b	223.0 ^f	335.4 ^b	383.1 ^d
T ₇ :T ₂ +vermicompost	242.5 ^a	326.1 ^a	330.4 ^c	396.8 ^b

Note: Mean values followed by the superscript in each column do not differ significantly at P=0.05 level by DMRT

DAC- Days after composting

CONCLUSION

From the present study it can be concluded that enzymatic activities can be considered as a descriptor of the biological stability of organic wastes. The microbial consortium found efficient in enhancing the degradation process and it can be used for hastening the process of silkworm pupal residue composting with agricultural waste. The enzyme activities found maximum in the treatment received silkworm pupal residue with silkworm litter and vermicompost. Hence, silkworm pupal residue with silkworm litter and vermicompost substrate combination is ideal for composting in order to enhance quality of matured compost.

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REFERENCES

- Casida, L. E., Klein, D. A. and Snatoro, T., (1964), Soil dehydrogenase activity. *Soil Sci.*, **98**: 371-376.
- Chonkar, P. K. and Tarafdar, J. C., (1984), Accumulation of phosphatases in soil. *J. Indian Soc. Soil Sci.*, **132**: 266-272.
- Eivazi, F. and Tabatabai, M. A., (1977), Phosphatases in soils. *Soil Biol. Biochem.*, **9**: 167-172.
- Frankken, B. W. T. and Dick. W. A., (1982), Relationships between enzyme activities during composting of waste microbial biomass from pharmaceutical industry. *Acta.Chim.Slov.*, **49**: 885-892.
- Garcia, C., Hernandez, T., Costa, F., Ceccanti, C. and Ganni, A., 1993, Hydrolases in organic matter fractions of sewage sludge: changes in composting. *Biores. Technol.*, **44**: 17-23.
- Garcia, J. C., Plaza, C., Rovira, P. S. and Polo, A., (2000), Long term effects of municipal solid waste compost application on soil enzyme activities and microbial biomass. *Soil Biol. Biochem.*, **32**: 1907-1913.
- Lasaridi, K.E. and Stentiford, E.I., (1998), A simple respirometric technique for assessing compost stability. *Water Res.*, **32**: 3717-3723.
- Nagaraj, G. and Basavanna, H. M., (1996), Proteins from silkworm pupae. *Silk worm info. Bull.*, **1**(1): 29-30.
- Pallab De, K., Arun, K. and De, S.K. (1990), Soil enzymes: Activities and agricultural importance. *Ind. J. Agril. Chem.*, **22**(2): 223 - 224.
- Patil, B. R., Singh, K. K., Pawar, S. E., Maarse, L. and Otte, J., (2009), Sericulture: An Alternative Source of Income to Enhance the Livelihoods of Small scale Farmers and Tribal Communities. Pro-Poor Livestock Policy Initiative. Res Rep. RR Nr. 09-03
- Pedrazzini, F. R. and Mckee, K. L., (1984), Effect of flooding on activities of soil dehydrogenase in rice. *Soil Sci. Plant Nutri.*, **30** (4): 359-365.
- Saviozzi, A., Cardelli, R., Levi -Minzi, R. and Riffaldi, R., (2004), Evolution of biochemical parameters during composting of urban wastes. *Compost Sci. Util.*, **12**(2): 153-160.
- Senthilraja, K., 2007, Formulation of suitable microbial consortium for composting and value addition of press mud fibre. *M.Sc. Thesis*, TNAU, Coimbatore, Tamil Nadu, India.
- Skujins, J., 1967, Enzymes in soil. In: Soil biochemistry (Eds.) A.D. McLaren and G. H. Peterson Marcel Dekker, New York. pp. 371 - 414.
- Solano, M. L., Iriarte, F., Ciria, P. and Negro, M. J., (2001), Performance characteristics of three aeration systems in the composting of sheep manure and straw. *J. Agric. Eng. Res.*, **79**(3): 317-329.

16. Speir, T. W. and Ross, D. J., (1978), Soil phosphatase and sulphatase. *In*: Burns, R.G. (ed.). *Soil Enzymes*. Academic Press, New York, pp. 197–250.
17. Tiquia, S. M., (2002), Evolution of extracellular enzyme activities during manure composting. *J. Appl. Microbiol.*, **92**: 764–775.
18. Vuorinen, A. H., (2000), Effect of bulking agent on acid and alkaline phosphor-monoesterase and D-glucosidase activities during manure composting. *Biores. Technol.*, **75**: 113–138.
19. Yashoda, K. P., Sachindra, N. M., Rao, D. N. and Mahendrakar, N. S., (2001), Changes in microbial population during fermentation of silkworm pupae. *Sericologia*, **41**(2): 253-261.
20. Zantan, M. L. and Bremner, J. M., (1976), Comparison of method of assaying urease activity in soils. *Soil Biol. Biochem.*, **7**: 1291-1295.

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