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# Comparative assessment in enzyme activities during vermicomposting of bio-degradable wastes

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**Abstract**
The present investigation was carried out to find out the changes in enzyme activities during vermicomposting which offers a promising solution for assessment of quality of vermicompost. The different bio-degradable wastes (agricultural wastes, kitchen wastes, vegetable market wastes and paddy straw) were composted separately using earthworms. The results revealed that initial enzyme activity (urease, acid, alkaline phosphatase and dehydrogenase) was high. With time, enzyme activity decreased as availability of organic compounds decreased. Highest enzyme activity was recorded with vermicompost prepared out of agricultural wastes while least with paddy straw.

**Keywords:** vermicompost, bio-degradable wastes, urease, acid phosphatase, alkaline phosphatase, dehydrogenase

## 1. Introduction
In India, urban areas generate around 62 million tons of municipal solid waste per annum out of which around 40% - 60% is organic in nature which can be converted in to useful product (Planning commission report – 2014) [1]. The biological treatment of these wastes appears to be most cost effective and carry a less negative environmental impact. Poor soil management ensuring continued maintenance and build of soil fertility is indispensable for greater productivity from agricultural land. Due to energy crisis, prohibitive cost of fertilizers and poor purchasing power of small and marginal farmers, it is imperative to develop strategy to use organic wastes to its maximum potential with proper technology to meet shortage of fertilizers and for improving soil fertility. The biological decomposition of these wastes is mediated by variety of bio-chemical processes in which enzymes play a key role. Vermicomposting is a bio-oxidation and stabilization process of organic materials involving joint action of earthworms and micro organisms where in organic fraction of wastes is converted in to valuable soil amendment called vermicompost. During vermicomposting organic matter is oxidized and stabilized mainly because of increased decomposition and humification (Atiyeh *et al.,* 2002) [2]. However recycling of organic wastes into value added vermicompost mainly depends on the quality of end product. An important aspect of compost quality is the stability of the compost which relates to its microbial activity. Soil application of non stabilized compost may cause phytotoxicity and adversely affects the environment (Butler *et al.,* 2001) [3]. Different physical, chemical and biological parameters have been considered for monitoring the stabilization of organic matter in the process of vermicomposting. The most common parameters include C/N ratio, CEC, water soluble carbohydrates, bio-degradability index (Manna *et al.,* 2000) [4] and $CO_2$ evolution, water soluble organic carbon (Wu and Ma, 2001) [5]. In contrast to most of the analytical techniques used for evaluation of compost stability, enzymatic activity determination is easy, fast and relatively inexpensive (Mondini *et al.,* 2004) [6] and the enzyme activities can be a part of reliable measure of compost stability and maturity. Therefore the present work was taken up to study the bio-chemical activity indicated by the changes in the enzyme activity at progressive stages of vermicomposting using different organic wastes like agricultural wastes, kitchen wastes, vegetable market wastes and paddy straw.

## 2. Materials and Methods
The experiment was conducted during November 2014 at Loyola Academy Degree & PG College, Alwal, Secunderabad. The waste materials selected for the study were a) Vegetable market wastes (VMW) – putrefied and left over vegetables were collected from Rythu Bazar, Alwal, Secunderabad, b) Agricultural wastes (AW) – mainly weeds were collected from college farm, Loyola Academy Degree & PG College, Alwal, Secunderabad, c) Kitchen wastes (KW) – kitchen wastes were collected from hostels d) Paddy straw (PS) obtained from college farm, Loyola Academy Degree & PG College, Alwal, Secunderabad. The different wastes collected were air dried separately after cutting into small pieces. Five different beds (dimensions L x W x H – 15m x 1.5m x 0.6m) were made separately on ground. At the bottom of each bed, 4-5 cm layer of coconut coir was placed. The wastes were added layer wise and sprinkled with 10 percent cow dung slurry sufficient to wet the surface. Over this another layer of waste was spread along with cow dung slurry uniformly. This procedure was repeated in similar fashion to complete 200 Kg of raw materials. Then top of the bed was covered with cow dung slurry to prevent exchange of gases. The earthworms *Eisenia foetida* and *Eudrilus eugeniea* were released (@350 worms / $m^3$) in to the bed after 8-10 days of partial decomposition. The beds were covered with gunny bags to provide darkness to worms, protection from predators, retention of moisture and to maintain stability of temperature. Proper moisture content (40-50%) and suitable temperatures

(25-28ºC) were maintained throughout the process of composting by sprinkling water on the gunny bags covering the beds. Vermicomposting of all wastes was completed in 60 – 66 days. The samples were collected from each vermicompost bed at the time interval of 15, 30, 45 and 60 days after incubation. The completion of vermicomposting was indicated by aggregation of earthworms at the bottom of the bed. The waste to vermicompost ratio ranged from 1:0.4 i.e., 40 kg of vermicompost was obtained from 100 kg of raw organic waste material. Mature vermicompost was black, granular, light weight crumbly powder remained at top layers. When vermicompost became ready, gunny bags covering the beds were removed & sprinkling of water was stopped. Moist samples were analyzed for the enzymatic activities i.e., urease, acid, alkaline phosphatase and dehydrogenase. Urease activity was assayed by quantifying the rate of release of $NH_4^+$ from the hydrolysis of urea (Tabatabai and Bremner, 1972) [7]. The acid and alkaline phosphatase activity was assayed by quantifying the amount of p-nitrophenol released and expressed as μg of p-nitrophenol released $g^{-1}$ $h^{-1}$ (Tabatabai and Bremner, 1969) [8]. Dehydrogenase activity was assayed by quantifying the μg of TPF (2, 3, 5-triphenyl formazon) produced and expressed as $g^{-1}$ $h^{-1}$ (Casida *et al.*, 1964) [9].

## 3. Results and Discussion

### 3.1 Urease activity

Irrespective of the organic waste materials used in the study, urease activity which catalyses hydrolysis of urea to $CO_2$ and $NH_4^+$ showed a decreasing trend during the experiment. Maximum urease activity (237.4 μg $NH_4^+$ $g^{-1}$ $h^{-1}$) was recorded with vermicompost prepared from AW followed by vermicompost prepared from KW (217.7 μg $NH_4^+$ $g^{-1}$ $h^{-1}$), VMW (203.3 μg $NH_4^+$ $g^{-1}$ $h^{-1}$), PS (151.9 μg $NH_4^+$ $g^{-1}$ $h^{-1}$) (Fig-1). The variation in urease activity among vermicomposts obtained from different organic sources was due to variation in organic matter content of initial raw materials used (Pramanik *et al.,* 2007) [10]. It was observed that the total reduction of urease activity in all the vermicomposts under study ranged from 16.8% to 24.0%, however the reduction was high (59% to 76% of total reduction) during the initial 15 to 30 days of decomposition. The high initial activity of the enzyme was due to availability of easily degradable substances which resulted in high microbial activity. The decrease in the later stages was due to lack of availability of carbon compounds for micro organisms which lead to decrease in microbial biomass. (Benitez *et al.* 2005) [11] reported decrease in urease activity during vermicomposting of lignocellulosic olive waste. (Rama Lakshmi *et al.* 2014) [12] recorded decreased urease activity with increasing time during vermicomposting of vegetable market waste.

### 3.2 Acid and alkaline phosphatase activity

Both acid and alkaline phosphatase activities decreased during the process of vermicomposting. Among the various organic wastes studied, vermicompost obtained from AW recorded highest acid and alkaline phosphatase activity (200.0, 655.8 μg of p-nitrophenol released $g^{-1}$ $h^{-1}$) while lowest activity was recorded with vermicompost obtained from PS (155.5, 557.4 μg of p-nitrophenol released $g^{-1}$ $h^{-1}$) (Fig-2 & 3). It was observed a decrease of acid phosphatase and alkaline phosphatase activity in all the vermicomposts under study which ranged from 12.4% to 15.5% and 5.0% to 5.3% respectively. However it followed the similar reduction pattern during the initial 15 to 30 days of decomposition and it was as high as (52.4% to 65.2% of total reduction) and (51.6 % to 56.6% of total reduction) in acid and alkaline phosphatase activities respectively. Phosphatase is an enzyme of agronomic value because it hydrolyses compounds of organic phosphorus and transforms them into different forms of inorganic phosphorus which are assimilable by plants. Phosphatase activity is an indication of 'P' mineralization power by micro organisms. This enzyme is more relevant for the evaluation of composting process since it is synthesized by micro organisms only and does not originate from plant residues. High acid and alkaline phosphatase activities during the initial stages of vermicomposting may be attributed to the availability of organic phosphate compounds present in the raw materials. This inturn was consumed by microorganisms and earthworms during the process of decomposition. Higher phosphatase activity in vermicompost obtained from AW was due to higher phosphorus content in initial raw material which in turn resulted in higher microbial activity. Alkaline phosphatase activity of all vermicomposts was high compared to acid phosphatase activities. This was due to neutral pH range of vermicomposts at which alkaline phosphatase remained more active (Pramanik *et al.,* 2007) [10]. Variation in acid and alkaline phosphatase activities shown by composts prepared from different waste materials may be due to combination of variation in earth worms and microbial activity and differences in various organic phosphate compounds present in different materials (Zachariah & Chhonkar, 2004) [13]. An initial increase followed by rapid decrease in alkaline phosphatase was recorded during vermicomposting of pig slurry (Aira *et al.,* 2007) [14]. Decrease in phosphatase activity during composting was reported by several workers (Mondini *et al.,* 2004, Raut *et al.,* 2008, Tejada *et al.,* 2009, Iria Villar *et al.,* 2016) [6, 15-17].

### 3.3 Dehydrogenase activity

Dehydrogenase activity decreased during the process of vermicomposting of different organic waste materials used in the study (vermicompost prepared from AW > KW > VMW > PS) (Fig-4). Maximum dehydrogenase activity in the initial stages corresponds to maximum microbial activity due to presence of high amount of easily decomposable organic compounds. Dehydrogenase activity is related to group of enzymes which participate in the metabolic reactions producing energy in the form of ATP through the oxidation of organic matter, which is especially interesting in the composting process. Dehydrogenase has been considered as an indicator of overall microbial activity because it occurs intracellularly in all living microbial cells. Dehydrogenase activity is dependent on availability of substrate (Bansal & Kapoor, 2000) [18]. Barrena *et al.* (2008) [19] reported that maximum dehydrogenase activity (0.54 mg TPF g dry $matter^{-1}$ $h^{-1}$) was observed at the end of thermophilic stage or at the beginning of the mesophilic stage (days from 20 to 30) which gradually decreased in maturation stage during composting of organic fraction of municipal solid waste. Dehydrogenase activity has been studied in few works to monitor biological

activity of composting process (Benitez *et al.*, 1999, Benito *et al.*, 2003, Singh and Ganguly, 2005, Garg *et al.*, 2006, Ravi Kumar *et al.*, 2008, Kayikcioglu and Okur, 2011, Rama Lakshmi *et al.*, 2014) [20-25, 12].

Fig. 1-4 depicts the changes in urease, acid phosphatase, alkaline phosphatase and dehydrogenase activities during vermicomposting of organic wastes.

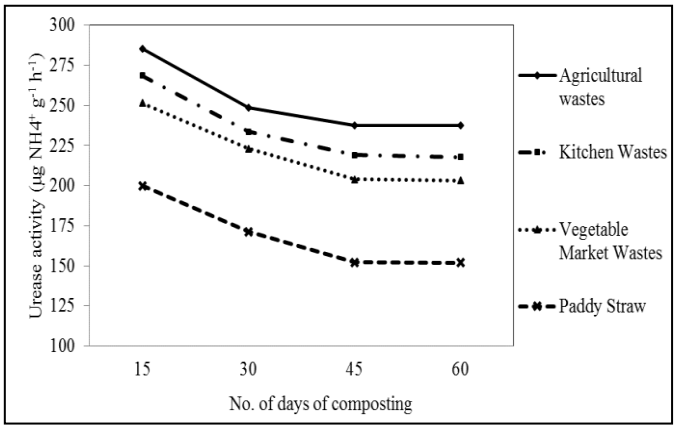


**Fig 1:** Changes in Urease activity during vermicomposting of organic wastes

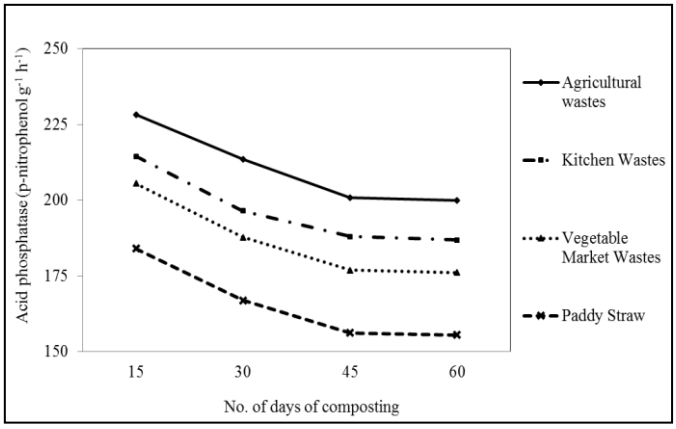


**Fig 2:** Changes in Acid Phosphatase activity during vermicomposting of organic wastes

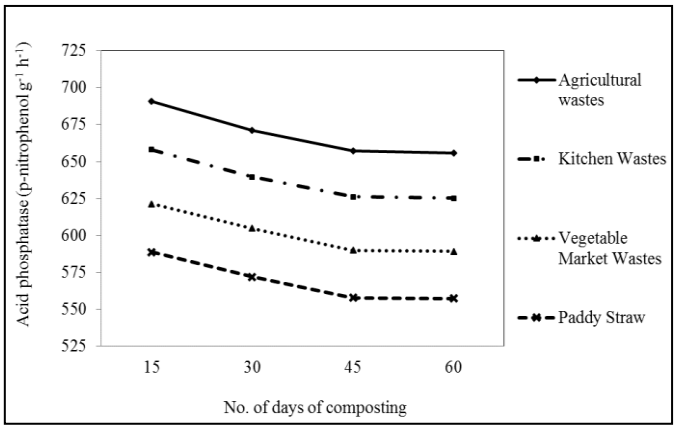


**Fig 3:** Changes in alkaline Phosphatase activity during vermicomposting of organic wastes

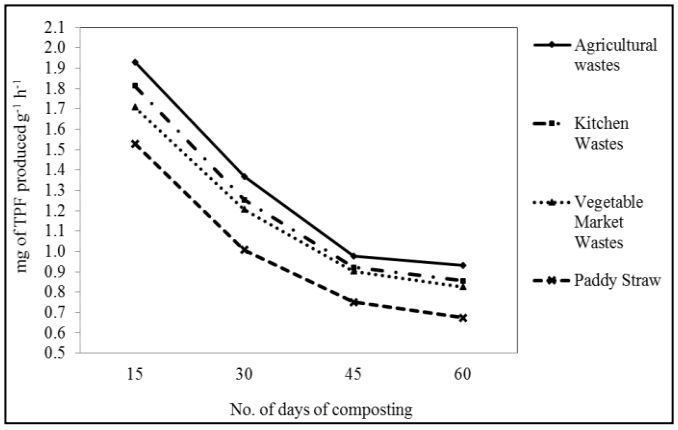


**Fig 4:** Changes in dehydrogenase activity during vermicomposting of organic wastes

## 4. Conclusion

During initial stage of vermicomposting, after earthworms get acclimatized to different substrates, the enzymatic activity was high due to degradation of easily available organic substances by free living microorganisms and those associated with earthworm's gut. In the later stages reduction in the enzymatic activity was due to lack of availability of carbon compounds for microorganisms which leads to decline in microbial biomass and this indicates stabilization of organic matter. In conclusion, assays of enzymatic activities proved to be useful method to describe biological activity of entire process of vermicomposting.

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