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Investigation of Enzyme Activities during Composting of Soiled Cotton Waste

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Authors' contributions

This work was carried out in collaboration between both authors. Author AP designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author LN managed the analyses of the study. Authors AP and LN managed the literature searches. Both authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Huge amount of soiled cotton waste is being produced by utilization of sanitary napkins by the women who have attained puberty. An attempt has been made to manage these wastes through composting and vermicomposting. Enzyme activities were studied during the composting and vermicomposting of soiled sanitary napkins. The highest protease (354.356 µg/g/hr), urease (0.104 µg/g/hr), amylase (119.08 µg/g/hr) and cellulase (170.13 µg/g/hr) activity was observed in vermicomposting sets. There was significant increase ($p<0.001$) in the enzyme activity in the vermicomposting and composting sets as compared to control.

Keywords: Composting; vermicomposting; urease; protease; amylase; cellulose.

1. INTRODUCTION

Soil is the important component of the ecosystem which is house of the diversified groups of organisms including flora and fauna. It is the laboratory for the process of decomposition that ultimately governs the availability of soil nutrients and its entry into plant biomass. Microbial

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decomposition pathways aided by suitable organic inputs play an important role in energy flow and nutrient cycling in the ecosystems [1,2]. Microbes such as bacteria, fungi, protozoa, actinomycetes have major role to play in the process of decomposition. The soil microbial biomass is one of the agents of transformation for added and native organic matter. Thus the soil microbial ecosystem plays an integral role in maintaining soil fertility through the biogeochemical cycling of essential plant nutrients and the mineralization of organic matter [3].

Man has attempted to control and directly utilize the process of decomposition for sanitary recycling and reclamation of organic waste material. Organic materials such as vegetable matter, animal waste and organic refuse can be converted from waste material to a form for use as a soil amendment by composting through (1) Anaerobic (without oxygen) decomposition, (2) Aerobic (with oxygen) decomposition and stabilization. In this process the saprophytic organisms feed upon decaying organic materials initially, and later on mites, millipedes, centipedes, springtails, beetles and earthworms help in transferring this into other soil layers. “Composting” is a controlled aerobic process carried out by successive microbial populations combining both mesophilic and thermophilic activities, leading to the production of carbon dioxide, water, minerals and stabilized organic matter [4]. Composting proceeds through three phases: mesophilic phase, lasting for a few days, is characterized by the activity and growth of mesophilic organisms, leading to a rapid increase in temperature. The thermophilic, or high temperature phase lasts from a few days to several months where thermophilic organisms dominate the degradation process. Finally, a several-month cooling and maturation phase occurs, characterized by the development of new mesophilic communities [5].

Vermicomposting is the process of utilizing earthworms for conversion of bio- degradable waste to compost. 60-70% enhancement in the natural biodegradation and decomposition occurs when earthworms are involved. Waste degradation by earthworms has proved to environmentally preferred technology [6]. Earthworms feed on the organic waste and they consume almost at the rate equal to their body weight [7]. They enhance the rate of decomposition by (i) increasing the rate of aerobic decomposition (ii) stabilizing the organic residues in them, (iii) enhancing the rate of functioning of the microbes and (iv) removing the harmful pathogens and heavy metals from the end products [6].

Rate of decomposition is determined by three major factors: soil organisms, the physical environment and the quality of the organic matter [8]. The microorganisms use the carbon as a source of energy and nitrogen for cell structure and growth. Successive decomposition results in the formation of humus by the process of humification. Humus affects soil properties, as it slowly decomposes, it colors the soil darker, increases soil aggregation and aggregate stability; increases the CEC (the ability to attract and retain nutrients); and contributes Nitrogen, Phosphorus and other nutrients. Decomposition of organic carbonaceous materials in soil is undertaken by groups of soil organisms acting in concert [3,8]. Carbonaceous materials subjected to microbial decomposition include cellulose, hemicelluloses and lignin. The organic matter is also classified on the basis of their rate of decomposition into (i) Rapidly decomposed: Sugars, starches, proteins etc. (ii) Less rapidly decomposed: Hemicelluloses, celluloses etc. (iii) Very slowly decomposed: Fats, waxes, resins, lignin and etc.

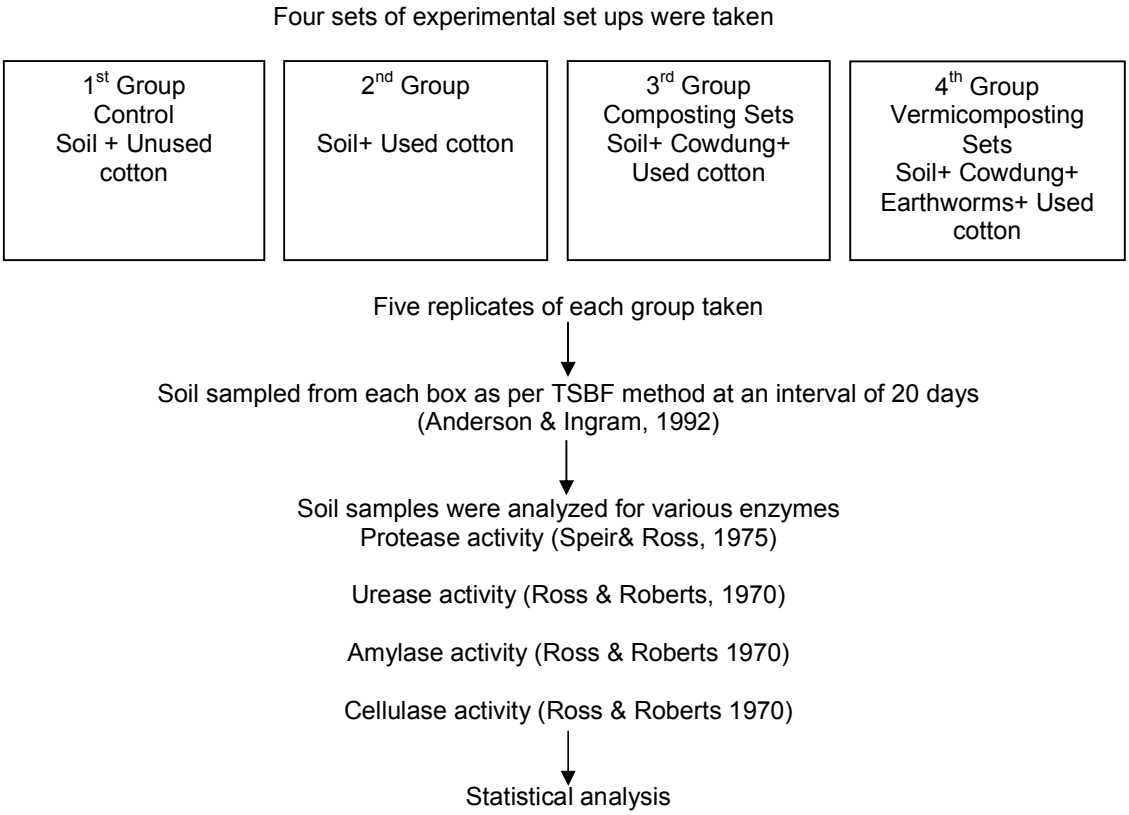
Microorganisms promote the degradation of organic materials through the activity of different hydrolytic enzymes [9]. Important enzymes involved in the composting process included cellulase, β-glucosidase, protease and xylanase, which depolymerize cellulose, hydrolyze glucosides, promote N-mineralization and hydrolyze xylan, respectively [10]. Characterizing and quantifying enzymatic activities during composting can reflect the dynamics of composting process in terms of the decomposition of organic matter and nitrogen transformations and may provide information about the maturity of composted products [2,11]. The enzymes secreted by various microorganisms during composting breakdown several complex organic compounds, finally leading to the formation of simple water-soluble compounds [12].

Cotton decomposition is very much important for present scenario. In India, the total cotton fiber consumption is estimated to be 26 lakh tons per year of which approximately 2, 10,000 tons of cotton dust is produced during yarn manufacturing process. An average woman uses 10 sanitary pads a month during her

menstruating years. In a span of about 35-40 menstrual years, she accumulates about 125 kilos of soiled pads. In India, there are about 355 million women of reproductive age. If all of them start using pads, they would be throwing away a mind- boggling 58,500 million used pads in a year. Used sanitary feminine napkins are usually subjected to landfill and dumpsites and it poses serious waste management problems [13,14]. However, used feminine napkins can be composted because its main components are wood pulp and non-woven cotton, which are compostable materials [15,16]. The disposal of the fabric materials used in textiles [15,17] is a serious challenge to waste management. Conventional methods for fabric waste management include land-filling, recycling and incineration. Most of them are disposed of by burning, which in turn increase carbon dioxide level in the atmosphere which adds onto global warming. This can be hazardous and thus needs an alternative remedy. In this study, vermicomposting and composting method has been selected for cellulosic waste management.

2. MATERIALS AND METHODS

2.1 Workflow to Study Composting and Vermicomposting of Soiled Cotton Wastes



2.2 Experimental Set up

Plastic boxes with one layer of gravel at the base were divided into four groups, each group with five replica i.e.

1st group (Control):- soil + unused cotton.
2nd group: - soil + used cotton.
3rd group: - soil + 10% cow dung + used cotton.
4th group: - soil + 10% cow dung + earthworms + used cotton.

2.3 Urease Activity

5 gm of soil was placed in a 50 ml flask to which 0.2 ml toluene followed by 9 ml of Tric-Hcl buffer was added and flask was swirled for few seconds and then 1 ml of 0.2 m urea was added. Again after little swirling the flask was stopper and placed in an incubator for 2 hrs at 37°C. Stopper was then removed and volume was made up to the mark (50 ml) by addition of KCl - $AgSO_4$ solution. The content was centrifuged and the supernatant was used for estimation of ammonia. Color was developed by adding 1 ml of phenate solution followed by 1 ml of alkaline hypochlorite solution to 1 ml supernatant. The reaction was allowed to proceed for 5 minutes at 37°C. 7 ml of distilled water was added and absorbance was measured at 625 nm. Controls were maintained when 1 ml of 0.2 m urea was added before the addition of KCl - $AgSO_4$ solution. Ammonium chloride was used as standard [18].

2.4 Protease Activity

2 gm of soil was placed in a 50 ml conical flask to which 0.2 ml toluene was added with 10 ml Tris containing sodium caseinate and incubated for 2 hrs at 30°C whereas control contained no sodium caseinate. 4 ml of trichloroacetic acid was added and the mixture was centrifuged. 2 ml of supernatant was treated with 3 ml of 1.4 M Na_2CO_3 and with rapid swirling 1 ml of Folin-ciocalteus reagent and absorbance was measured after 30 minutes at 700 nm. Tyrosine was used as standard [18].

2.5 Amylase Activity

3 gm soil was placed in a 50 ml flask to which 0.2 ml toluene was added and allowed to stand for 15 minutes at room temperature. 6 ml Sorenson's buffer and 6 ml substrate solution (1% soluble starch) was added, after little swirling it was placed in an incubator at 30°C. Control was maintained by addition of distilled water instead of substrate. After 24 hr the content of the flasks was centrifuged. 2 ml of 3, 5 dinitrosalicylic acid (color reagent) to 1 ml supernatant was added, color was developed after keeping the solution for 5 minutes in boiling water and absorbance was measured at 540 nm [19].

2.6 Cellulase Activity

3 gm soil was placed in a 50 ml flask to which 0.2 ml toluene was added and allowed to stand for 15 minutes at room temperature. 6 ml Sorenson's buffer and 6 ml substrate solution (3% CMC) was added, after little swirling it was placed in an incubator at 30°C. Control was maintained by addition of distilled water instead of substrate. After 24 hr the content of the flasks was centrifuged. 2 ml of 3, 5 di-nitro salicylic acid (color reagent) to 1 ml supernatant was added, color was developed after keeping the solution for 5 minutes in boiling water and absorbance was measured at 540 nm [19].

2.7 Statistical Analysis

The results obtained were subjected to two way ANOVA using MS Excel 2007 'Data Analysis Tool'.

3. RESULTS AND DISCUSSION

3.1 Protease Activity

Initially the protease activity was (12.88 µg/g/hr). Maximum protease activity was found on 20^{th} day in all four groups then it gradually decreased till 60^{th} day except for control (Group A) and Group C (Fig. 1 and supplemental 1). The highest protease activity was observed in Group D (354.356 µg/g/hr). Two way ANOVA shows significant difference ($p<0.001$) between the treatment as well as duration.

Protease activity increased gradually then decreased except in one experimental set in which it increased. Proteases are considered as indicators of decomposition process. DELA Horra et al. in 2005 [20] found same results during composting of horse and poultry manures with wheat straw. Protease activity in different sets may be attributed due to breakdown of complex nitrogen compounds to more simple compounds [21]. Castaldi et al. in 2008 [22] found that protease activity was strongly correlated with water soluble nitrogen forms.

3.2 Urease Activity

At the beginning of the process urease activity was found to be (0.104 µg/g/hr). Generally Urease activity increased at the end of the composting process in all four Groups except for Group B. The maximum urease activity was observed in Group D (0.885 µg/g/hr) by 60^{th} day (Fig. 2 and supplemental 2). There was significant increase ($p<0.001$) in the urease activity in the vermicomposting and composting sets as compared to control.

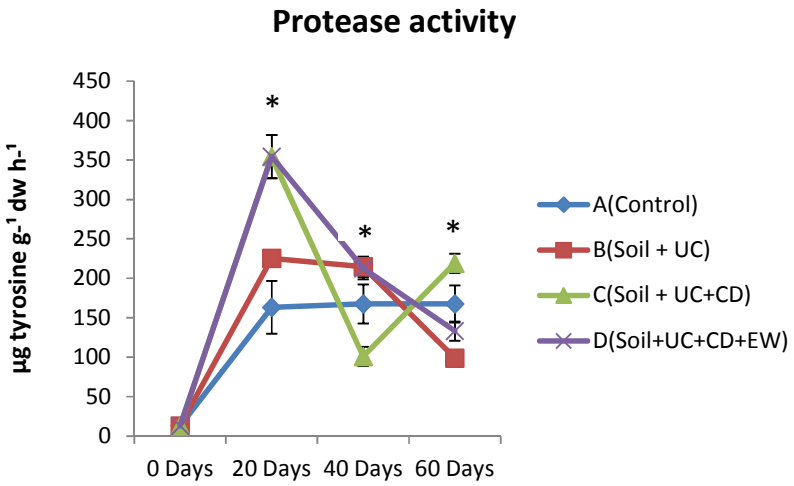


Fig. 1. Protease activity of soil during the composting of soiled waste with respect to treatment and duration. There are significant differences between (A, B, C and D) with respect to treatment and duration. Five replicates have been carried out for each experiment; standard deviations have been represented; Asterisk mark shows significant differences at 0.001 level of significance

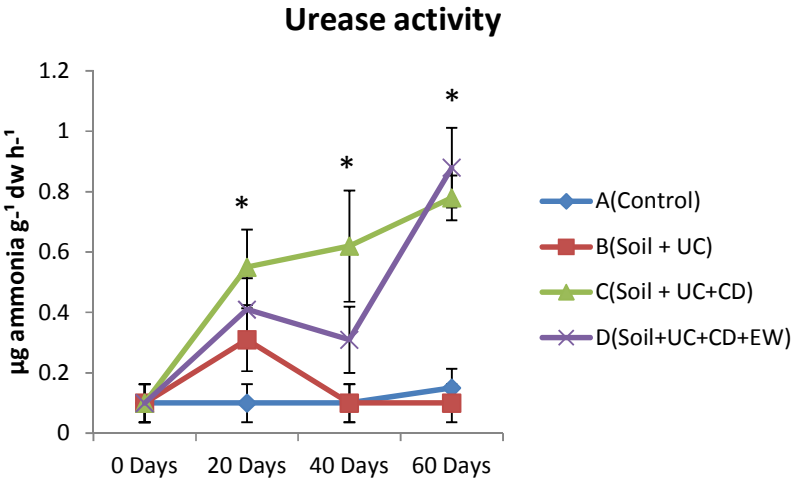


Fig. 2. Urease activity of the soil in different treatment with respect to duration. There are significant differences between (A, B, C and D) with respect to treatment and duration. Five replicates have been carried out for each experiment; standard deviations has been represented; Asterisk mark shows significant differences at 0.001 level of significance

Urease activity increased in all experimental set except one which may be due to availability of water soluble nitrogen [23]. Urease activity is involved in the hydrolysis of urea to ammonia and carbon dioxide [22].

3.3 Amylase Activity

At initial stages amylase activity was found to be (9.92µg/g/hr). In Group B, C, D it gradually increased up to 40th day then it again decreased in all Groups except for Group B (Fig. 3 and supplemental 3). The highest amylase activity was found in Group D (119.08 µg/g/hr) on 40th day. There was significant difference ($p<0.001$) in the amylase activity in the vermicomposting and composting sets as compared to control.

Amylase activity increased gradually and then decreased at the end of the process. Maximum

amylase activity has been found in vermicomposting on 40th day. This may be due to the increase in enzymatic activity of the microorganisms inside the earthworm's gut. Enhanced microbial activity inside the earthworm gut has also been reported by various workers [24,25].

3.4 Cellulase Activity

Cellulase activity was (31.72 µg/g/hr) at the beginning. It gradually increased up to 40th day then it again decreased on 60th day except for Group C (Fig. 4 and supplemental 4). The maximum cellulase activity was found in Group D on 40th day (170.13 µg/g/hr). Two way ANOVA showed significant difference ($p<0.001$) in cellulase activity with respect to treatment as well as duration.

Cellulase activity was high at the beginning of the process but decreased gradually as the decomposition progressed which may be due to degradation of organic matter. Similar results were obtained by Li et al. [26].

Qualitative and quantitative presence of extracellular enzymes are associated with the process of decomposition and mineralization of organic matter [27-29]. The most important indicators of soil microbial activity in the soil include the activity of extracellular hydrolytic enzymes which are involved in nutrient cycling [30]. In the present study the enzymatic activities have been found to be increased in vermicomposting sets. The highest enzymatic activity in the vermicomposting sets indicates higher microbial activity in presence of earthworms. Earthworms facilitate the activity of microbes by altering the physical and chemical properties of the soil [6,24,25]. Other studies also indicate that the evaluation of enzymatic activities can be utilized as indicators to detect changes resulting from agricultural management practices [30-33].

The amendment of cowdung (in Group C) and inoculation of earthworms (in Group D) resulted in higher rate of decomposition as indicated by the enhanced enzymatic activity. Similar results have been reported by Garcia et al. [34]. Increases in β-glucosidase activity after compost application consisting of municipal solid residues were also reported by Marcote et al. [35] and Ros et al. [36], indicating the effect of the substrate. Studies indicate increases in phosphatase activity resulting from organic matter amendments [37,38].

The increased enzyme activity, an indicator of microbial activity related to the cycling of chemical elements, significantly increased after cowdung and earthworm amendment, compared to the control may be due to increased microbial biomass, which may have produced these enzymes [39-42].

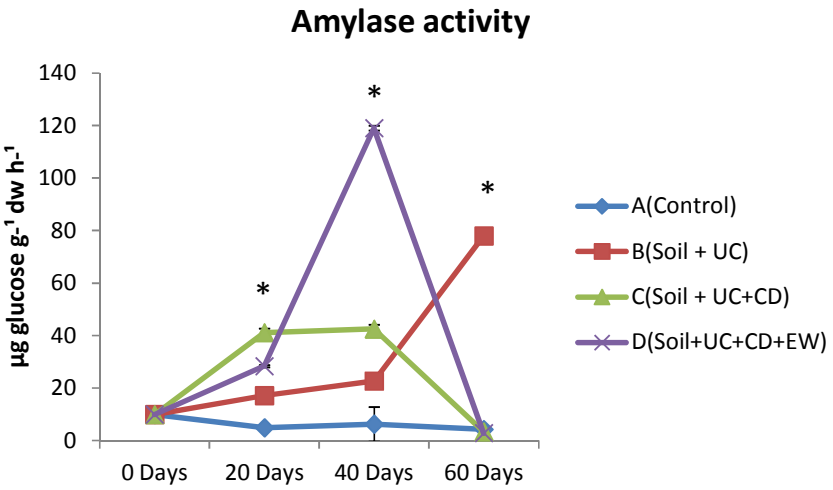


Fig. 3. Changes in amylase activity of soil during the composting of soiled waste with respect to treatment and duration. There are significant differences between (A, B, C and D) with respect to treatment and duration. Five replicates have been carried out for each experiment; standard deviations has been represented; Asterisk mark shows significant differences at 0.001 level of significance

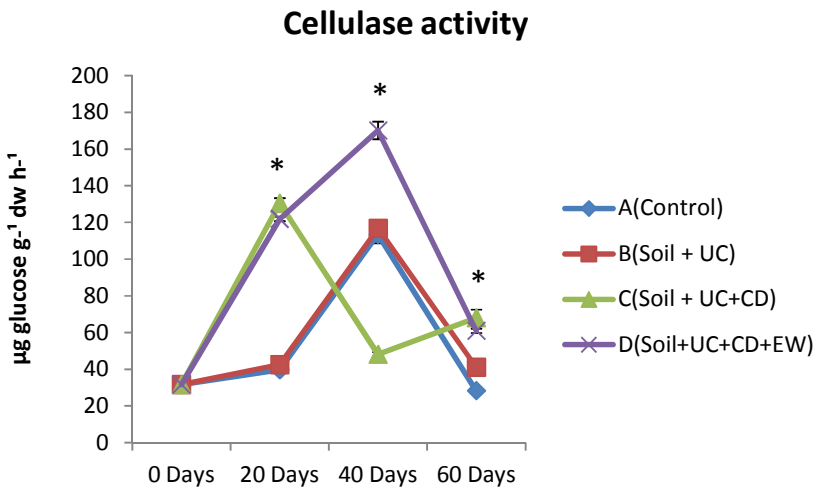


Fig. 4. Cellulase activity of soil during the composting of soiled wastes with respect to treatment and duration. There are significant differences between (A, B, C and D) with respect to treatment and duration. Five replicates have been carried out for each experiment; standard deviations has been represented; Asterisk mark shows significant differences at 0.001 level of significance

4. 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. As soiled wastes take a longer period of time to decompose it can be processed by the means of composting and vermicomposting and converted into organic fertilizer. It can be considered as alternative remedy for reducing or eliminating organic waste land filling (in which anaerobic decomposition takes place releasing methane as by product), thereby preventing environmental pollution as aerobic decomposition releases CO_2 and water as by products. Although CO_2 has been identified as the most responsible gas for global warming (water vapour is also a greenhouse gas) but when compared with pollution intensity, methane (CH_4) is a more vulnerable polluter than CO_2 as it 21 times intensifies global warming than CO_2. Therefore, release of CO_2 in composting and vermicomposting processes is a somewhat environmentally friendly practical solution than methane emittance.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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