

## Analysis and advanced characterization of municipal solid waste vermicompost maturity for a green environment

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### Abstract

Rapid demographic expansion along with increasing urbanization has aggravated the problem of solid waste management. Therefore, scientists are seeking waste management methods that are eco-friendly, cost effective and produce immediate results. In the developing world, municipal solid waste (MSW) contains mostly organic substances, therefore vermicomposting could be a better and cost-effective option for waste management. In this study, vermicomposting of organic portion of MSW with cow dung (additive) was performed using *Eisenia fetida*. The results showed significant ( $p < 0.001$ ) decline in pH (13.17%), TOC (21.70%), C: N (62.53%) and C: P (57.66%) ratios, whilst total N (108.9%), P (84.89%) and K (21.85%) content increased ( $p < 0.001$ ) in matured vermicompost. Different enzymatic activities declined during termination phase of vermicomposting experiments with maximum decrease of 41.72 ( $p = 0.002$ ) and 39.56% ( $p = 0.001$ ) in protease and  $\beta$ -glucosidase, respectively. FT-IR, TGA, DSC and SEM studies suggested that final vermicompost was more stabilized as compared to initial waste mixture, characterized by reduced levels of aliphatic materials, carbohydrates and increase in aromatic groups possibly due to biosynthesis of humic substances. Both, the conventional (physicochemical and enzyme activity) and advanced techniques depict maturity and stability of the ready vermicompost. However, FT-IR, TGA, DSC and SEM were proved to be more promising, fast and reliable techniques over conventional analyses.

**Keywords:** Municipal solid waste (MSW); vermicompost; *Eisenia fetida*; spectroscopy; thermogravimetry (TG); differential scanning calorimetry (DSC)

## 1. Introduction

Prodigious population growth and urbanization along with techno-economic development are responsible for continuing expansion of cities. It has put additional pressure on infrastructural services and food security across the globe (Srivastava et al., 2015). These factors affect the consumption pattern of the society that has been accompanied by high amount of solid waste generation and its compositional changes (Khan et al., 2016). As stated in the World Bank report (2018), the annual rate of municipal solid waste (MSW) generation were 2,010 million tonnes in 2016, which is projected to reach 3,400 million tonnes by 2050. A huge quantum of global waste (33%) usually finds its way to open dumping sites which is still the preferable method for waste disposal in low income countries (93%). About 75% of the solid waste is open dumped in South Asia, followed by Sub-Saharan Africa (69%) and Middle East and North Africa (52.7%). In low- and middle-income countries, the quantum of organic fraction of waste is exceeds 50% which upon degradation produces various noxious gases (e.g. CH<sub>4</sub>, CO<sub>2</sub>, and H<sub>2</sub>S) and leachate at dumpsites that poses serious environmental and health hazards (Goel et al., 2017; Sharma et al., 2018). Therefore, it is imperative to identify simple techniques for solid waste reduction and stabilization with low operational and maintenance cost that would also help in achieving the environmental and public health standards (Srivastava et al., 2016; Goel et al., 2017). Additionally, segregation of organic fractions will lead to less amount of waste reaching dump sites.

Nowadays, agricultural application of organic waste is getting recognition across the globe due to high proportion of organic matter and nutrient rich profile, which also reduces dependency over inorganic fertilizer (Srivastava et al., 2016). However, processing of these organic wastes is obligatory prior to its agricultural utilization (Srivastava et al., 2018). Recently, vermicomposting of organic waste has received research attention globally and is considered as promising and sustainable technology to reuse organic wastes. Vermicomposting is a non-thermophilic exercise which transform complex organic materials into a nutrient rich stabilized product through bio-oxidation (Srivastava et al., 2015; Lim et al., 2016). In vermicomposting, degradation of organic waste materializes through gut microbes of earthworm and results in a stable and mature vermicompost (Lim and Wu 2015; Lim et al., 2016). Stable vermicompost is humus like material, having nutrient rich profile and plant growth hormones which can augment soil physico-chemical and microbial properties and enhance plant growth (Srivastava et al., 2016; Bhat et al., 2017).

The agricultural use of organic waste manure produced through vermicomposting is one of the possible approaches to restore soil productiveness and organic waste management (Ostos et al., 2008; Bhat et al., 2016). However, it is essential to determine vermicompost stability before its

agricultural applications that may otherwise cause phytotoxicity (Aamir et al., 2010). Stability is directly linked to biological activity of vermicompost and is dependent on degree of biodegradation achieved during vermicomposting process, while maturity is associated to phytotoxin free final vermicompost (Wang et al., 2004). In addition to the alterations in physico-chemical and enzymatic activities, the stability and maturity of vermicompost can also be determined by spectroscopic and thermogravimetric methods (i.e. fourier transform infrared (FT-IR) spectroscopy, differential scanning calorimetry (DSC) and scanning electron microscopy (SEM)) ( Amir et al., 2010; Ravindran et al., 2013; Hussain et al., 2015; Lim and Wu, 2016; Soobhany et al., 2017; Khatua et al., 2018). Previous studies have shown that FTIR, TG and DSC analyses are among the most promising, fast and reliable techniques for characterizing heterogeneous organic material, which provide a complete information on compositional, functional and behavioral properties of samples (Kumar et al., 2013; Ouaquoudi et al., 2015; Soobhany et al., 2017). Likewise, SEM analysis gives information on morphological structure of vermicompost that validates maturity and stability of product (Rajpal et al., 2014; Bhat et al., 2015; Hussain et al., 2016).

Earlier studies on vermi-stabilization of different organic wastes focused on changes in physico-chemical properties ( Suthar et al., 2015; Hussain et al., 2016; Arora and Kaur, 2019), enzyme dynamics (Yadav et al., 2015; Villar et al., 2016; Sudkolai and Nourbakhsh, 2017) and evaluation of vermicompost as soil organic amendments ( Lakhdar et al., 2012; Weber et al., 2014; Srivastava et al., 2018). To date very limited studies are present which include both traditional and advance techniques to evaluate stability and maturity of organic waste through vermicomposting. Also, the information of the appropriateness of vermitechnology for transmuting organic portion of MSW into humic substances and qualitative analysis of vermicompost is still in its infancy. Therefore, in this study multiple techniques were used to evaluate stability and maturity of vermicompost with following objectives: (a) analysis of the physico-chemical changes during vermicomposting process of organic portion of MSW mixed with cow dung; (b) evaluation of enzymatic dynamics in initial feed material and final vermicompost; (c) compositional, functional and structural changes in initial feed material and final vermicompost using FTIR, TGA, DSC and SEM.

## **2. Material and methods**

### *2.1. Experimental setup, vermicomposting of municipal solid waste, and quality control*

The experiment was carried out at the test site of Institute of Environment and Sustainable Development (IESD), Banaras Hindu University, Varanasi, during October to December 2016. The mean monthly maximum temperatures ranged between 21.2 to 32.2°C and mean monthly minimum temperatures between 11.3 to 21.0°C during the study period. Total precipitation was recorded to 5.4 mm while mean monthly maximum and minimum relative humidities varied from 76.4 to 93.2% and 45.0 to 68.0 %, respectively. The substrate was prepared by mixing dried chopped organic portion of municipal solid waste (OPMSW) (containing vegetable, paper, flower, leaf litter etc.) and cow dung (CD) in ratio of 60: 40 (w/w). The OPMSW is heterogenous and complex in nature having various components with different physico-chemical properties. Moreover, microbial activation is essential to initiate biodegradation of OPMSW. Many previous studies suggest that CD is a suitable bulking material for making a microbially active substrate with different organic waste components during the vermicomposting process (Paul et al., 2011; Suthar et al., 2015; Srivastava et al., 2018). Apart from that, CD addition is well known to improve the stabilization process of different waste stuffs and increase of the earthworms' survival rate (Lim et al., 2016; Yuvaraj et al., 2018). Therefore, CD appeared to be a suitable bulking material with OPMSW for preparing the substrate. Substrate material was pre-decomposed for 3 weeks and thorough turning was performed after each week. The moisture level was maintained by spraying water during this period. Pre-decomposition was performed to attain thermal stability and the removal of volatile toxic substances (Srivastava et al., 2015; Arora and Kaur, 2019; Sharma and Garg, 2019). Also, it helps in making the substrate material palatable to the earthworms and breaking oily and fatty substances in the feed material prior to the earthworm inoculation (Sharma and Garg, 2019). The physico-chemical characteristics of pre-decomposed CD, OPMSW and waste mixture are presented in **Table 1**. After pre-decomposition; healthy, equal sized and non-clitellated 25 earthworms (*Eisenia fetida*) were hosted in plastic containers of 2 litre capacity containing 1000 gm of mixed substrate. The setup was established in triplicate. The moisture level in the substrate was kept at 70-80% by sprinkling fresh water on alternate days. The samples were collected from all the three established vermicomposting units in triplicate. These sub-samples from all three vermicomposting units (total 9 nos.) were mixed to form single composite sample to maintain homogeneity and to make a representative sample. The representative sample was divided into three parts (for initial and final sampling). The first part was air-dried in shade at room temperature till stable mass was achieved. This was followed by physico-chemical analyses. Second part was air dried for 3-4 hours in shade at room temperature to remove excess moisture content attained during vermicomposting and

refrigerated in the plastic vials below 4°C to keep samples stable until further instrumental analyses (Ernesto et al., 2009; Wu et al., 2011; Kumar et al., 2015). The third part was not air-dried and directly kept in plastic vials at 4°C for different enzyme analyses. The physico-chemical analyses were performed at every two-week interval throughout the vermicomposting operation, while different instrumental, and enzyme activity were measured in initial substrate and final vermicompost samples.

Quality control was assured by the use of analytical grades (AR) chemicals, standard protocols and operating procedures, and calibration with standards. IBM Statistical Package for the Social Sciences (SPSS) version 16 (Illinois, USA) was applied for all the statistical analyses. Sigma Plot version 10 software was used for all the graphs related to different enzyme analysis, while Origin 9.1 software was used to plot the graphs for spectroscopic and thermal analyses.

**Table1** Initial physico-chemical properties of pre-decomposed CD, OPMSW and waste mixture (mean  $\pm$  SE, n = 3)

Parameters	Pre-decomposed material (3 weeks)		
	CD	OPMSW	Waste mixture
pH	7.71 $\pm$ 0.003	8.58 $\pm$ 0.003	8.35 $\pm$ 0.01
EC (mS cm <sup>-1</sup> )	1.43 $\pm$ 0.01	1.29 $\pm$ 0.01	1.27 $\pm$ 0.01
TOC (g Kg <sup>-1</sup> )	425.1 $\pm$ 0.50	509.8 $\pm$ 4.10	465.9 $\pm$ 0.99
TKN (g Kg <sup>-1</sup> )	6.24 $\pm$ 0.12	10.54 $\pm$ 0.17	8.06 $\pm$ 0.12
Total P (g Kg <sup>-1</sup> )	5.41 $\pm$ 0.25	8.86 $\pm$ 0.12	7.81 $\pm$ 0.12
Total K (g Kg <sup>-1</sup> )	10.06 $\pm$ 0.29	13.89 $\pm$ 0.01	13.27 $\pm$ 0.12
C/N ratio	68.16 $\pm$ 1.41	48.40 $\pm$ 0.94	57.81 $\pm$ 0.95
C/P ratio	78.97 $\pm$ 3.80	57.54 $\pm$ 0.72	59.69 $\pm$ 0.83

## 2.2. Physico-chemical analyses of vermicompost

Measurements of electrical conductivity (EC, Model 303, Systronics, India) and pH (Model 802, Systronics, India) were achieved with suspension of vermicompost in distill water (ratio of 1:10 (w/v)) that has been mixed thoroughly for 30 min with subsequent filtration via filter paper (Whatman No. 1). The total organic carbon (TOC) was estimated by ‘dry combustion method’ as described by Nelson and Sommers (1982) (**Appendix S1**). Total nitrogen (N) content of samples was ascertained using automatic N analyzer (Model KEL PLUS, India) by applying Kjeldahl method (Jackson, 1975). Total phosphorus (P) was worked out using procedure prescribed by Allen et al. (1974), and potassium (K) content was deduced by using flame photometric method. Analysis of variance (ANOVA) was implemented to decide the significant differences between different time intervals for the observed physico-chemical characteristics of vermicompost using Duncan’s multiple range test (DMRT) post hoc test.

### 2.3. Enzyme activities

The urease enzyme activity was ascertained by ammonium released. Measurement were conducted after nurturing the samples (5 g fresh weight) with urea solution (0.08 M) for 2 hours at 37 °C along with KCl solution (1N KCl in 0.01 N HCl), at 690 nm (Kandeler and Gerber, 1988). The acidic phosphomonoesterases activity was assessed by the release of p-Nitrophenol (PNP). For this purpose, samples (1 g fresh weight) were incubated (1 h @ 37 °C) in presence of p-nitrophenyl phosphate (0.025 M) and measurement at 400 nm (Eivazi and Tabatabai, 1972). The dehydrogenase enzyme activity was inferred by evaluation of the rate of decrease of triphenyl tetrazolium chloride (TTC) (3%) to triphenyl formazan (TPF). Quantification was done at 485 nm after incubation at 37 °C for 24 hours (Casida et al., 1964). Estimation of protease activity was accomplished by measuring tyrosine extricated. For this, samples (1 g fresh weight) were incubated (2 hrs @ 50 °C) in presence of sodium caseinate (2%) and with Folin–Ciocalteu reagent, at 700 nm. The  $\beta$ -Glucosidase activity was analyzed by determining p-Nitrophenol (PNP). This was determined by incubating (1 hour @ 37 °C) samples (1 g fresh weight) using p-Nitrophenyl  $\beta$ -D-glucopyranoside (PNG, 0.025 M), at 400 nm (Eivazi and Tabatabai, 1988). Paired Student's t-tests were employed to find the significant change among enzymatic activities in initial waste mixture and final vermicompost.

### 2.4. Fourier transform infrared (FT-IR) analysis

The Fourier transform infrared (FT-IR) study was performed on a Nicolet™5700 spectrometer. 2 mg of each samples were assorted with 400 mg KBr and pressed under vacuum to make potassium bromide (KBr) pellets. Both samples and KBr were dehydrated discretely at 105°C to remove moisture before production of the pellets. The bands measured were ranged from 400 to 4000  $\text{cm}^{-1}$  with interval of 4  $\text{cm}^{-1}$ . Baseline corrections were made in spectra and absorbance was normalized for unity of presentation and analysis.

### 2.5. Thermogravimetric (TGA) analysis

Thermogravimetric analysis (TGA) was accomplished using a simultaneous thermal analyzer (PerkinElmer STA 600, USA). The samples were grounded (15-25 mg) and sieved to 0.2 mm particle size. The analysis was completed on a dry basis by means of aluminum crucibles (60 $\mu$ L) under synthetic air atmosphere (flow rate maintained at 100  $\text{ml min}^{-1}$ ). Temperature ranged from 20 – 800 °C and heating rate of 10 °C  $\text{min}^{-1}$  was used with pressure sustained at 101 kPa.

## 2.6. Differential scanning calorimetry (DSC) analysis

Differential scanning calorimetry (DSC, Mettler Toledo 822E, India) was carried out on finely ground samples. Covered aluminum crucibles were used for weighing samples (8 - 10 mg). Thermal analysis was accomplished in reduced nitrogen atmosphere (temperature range: 0–220 °C, heating rate 10 °C/min).

## 2.7. Scanning electron microscopy (SEM) analysis

Scanning electron microscopy (SEM) of vermicompost samples was conducted through a scanning electron microscope (Nova NanoSEM 450, Czech Republic). The samples prepared with glutaraldehyde were attached on specimen holder. The mounts were dried under a high vacuum and coating was performed. Scrutiny of these specimens was made at 4000X magnifications.

# 3. Result and Discussion

## 3.1. Changes in physico-chemical properties

In this study, the final vermicompost was more stabilized and having nutrient rich profile than initial substrate (**Table 2**). The physico-chemical properties of final vermicompost were found to differ considerably as equated to initial substrate. The pH of the substrate decreased significantly (ANOVA;  $F=3,098$ ,  $p<0.001$ ) through progression of vermicomposting process and it was 7.25 in final vermicompost as compared to 8.35 in initial substrate. The decline in pH may be credited to the production of carbon dioxide (CO<sub>2</sub>), ammonia (NH<sub>3</sub>), nitrates (NO<sub>3</sub><sup>-</sup>), orthophosphates (PO<sub>4</sub><sup>-3</sup>) and organic acids during bioconversion process (Sharma and Garg, 2017, 2019; Yuvaraj et al., 2018; Karmegam et al., 2019). During the vermicomposting process, carboxylic and phenolic groups of humic acids (HAs) were neutralized by intestinal Ca and NH<sub>3</sub> secreted by earthworms (Sharma and Garg, 2017, 2018). Consequently, the pH of final vermicompost tended toward neutral range. In the present study, lowering of pH during vermicomposting process assisted to conserve nitrogen in final vermicompost (Sharma and Garg, 2017; Arora and Kaur, 2019). The electrical conductivity (EC) increased significantly (ANOVA;  $F=4,725$ ,  $p<0.001$ ) from 1.27 to 2.79 mS cm<sup>-1</sup> in initial substrate and final vermicompost, respectively. The rise in EC was due to degradation of organic matter and elevated levels of soluble mineral salts in available forms during vermicomposting (Lim et al., 2011; Sharma and Garg, 2017, 2018; Karmegam et al., 2019). Total organic carbon (TOC) significantly decreased by 21.70% in final vermicompost in comparison to initial waste mixture

(**Table 2**) (ANOVA;  $F=177.76$ ,  $p<0.001$ ). This trend of TOC loss can be ascribed to the organic matter degradation and mineralization. Also, respiratory activity and assimilation of carbon by microbial population and earthworms led to decrease in organic carbon (Sharma and Garg, 2017, 2018; Karmegam et al., 2019). The low level of TOC in final vermicompost showed humic acid richness indicating stabilization of the final product (Goswami et al., 2016; Sharma and Garg, 2017). Furthermore, increased level of total Kjeldahl nitrogen was noticed in the final vermicompost (16.84 g Kg<sup>-1</sup>) i.e. 108.9% as compared to initial waste mixture (8.06 g Kg<sup>-1</sup>) (ANOVA;  $F=414.71$ ,  $p<0.001$ ) (**Table 2**). Rise in TKN value may be ascribed to the mineralization of nitrogenous organic substrate (mainly proteins) and activity of N-fixers and microorganisms (Gómez-Brandón et al., 2013; Sharma and Garg, 2019). Further, excretory substances of earthworms such as body fluids, coelomic fluids (mucus), growth stimulating hormones, enzymes and dead earthworms augmented total nitrogen of final vermicompost (Arumugam et al., 2017; Sharma and Garg, 2017, 2018, 2019). Likewise, a significant increase of 84.89% was noticed in total phosphorus (TP) of final vermicompost (ANOVA;  $F=325.72$ ,  $p<0.001$ ) (**Table 2**). The increase in TP may be attributed to the mineralization of phosphorus by the activity of gut and faecal phosphatases, which were further complemented by P-solubilizing microbes during vermicomposting (Hanc and Pliva, 2013; Suthar et al., 2015; Sharma and Garg, 2017, 2018, 2019). Potassium (K) was also found to be higher during vermicomposting process and was increased from 13.27 to 16.17 g Kg<sup>-1</sup> in the initial substrate and final vermicompost respectively (ANOVA;  $F=52.03$ ,  $p<0.001$ ). Mineralization of K mainly occurs in the gut of earthworms through endo or exogenic enzymes (Yadav et al., 2013; Sharma and Garg, 2017, 2018, 2019). The C/N ratio shows the stability and maturity levels of compost/ vermicompost. In the present study C/N ratio significantly decreased by 62.53% (21.66) in final vermicompost as compared to initial substrate (ANOVA;  $F=583.90$ ,  $p<0.001$ ) (**Table 2**). The degradation and mineralization of organic matter and loss of carbon in respiratory activity and N enrichment through progression of vermicomposting process led to decreased C/N ratio in final vermicompost (Malafaia et al., 2015; Sharma and Garg, 2017, 2018, 2019). Also, increase in humification rate and decomposition of complex substances such as lignin, cellulose and hemicellulose resulted in reduced C/N ratio (Das et al., 2016; Sharma and Garg, 2017). As C/N ratio below 20 is a good indicator of vermicompost maturity (Morais and Queda, 2003), therefore vermicompost produced in the present (21.66) study could be considered as mature and stable. The C/P ratio of final vermicompost decreased by 57.66% and was found to be 25.27 as compared to 59.69 in initial substrate (ANOVA;  $F=781.91$ ,  $p<0.001$ ). The loss of carbon and increase in phosphorus content during vermicomposting



operation led to reduced C/P ratio in vermicomposted material (Sharma and Garg, 2017; Karmegam et al., 2019). Based on the nutrient profile, cow dung spiked organic portion of MSW could be recommended for the production of vermicompost, however it relies on the physico-chemical properties of organic waste. It is well known fact that NPK are essential nutrients that augment plant growth and productivity. Therefore, the nutrient rich profile of MSW vermicompost, proved its appropriateness for agricultural application. Hence, it can be stated that vermicomposting of OPMSW has immense potential for recycling and recovery of plant nutrients from wastes.

**Table 2** Physico-chemical properties of substrate material at different time-intervals during vermicomposting (mean  $\pm$  SE, n = 3)

Parameters	0 days	14th day	28th day	42nd day	56th day	70th day	84th day
pH	8.35 $\pm$ 0.01 <sub>a</sub>	8.12 $\pm$ 0.00 <sub>b</sub>	7.96 $\pm$ 0.01 <sub>c</sub>	7.63 $\pm$ 0.01 <sub>d</sub>	7.45 $\pm$ 0.01 <sub>e</sub>	7.37 $\pm$ 0.00 <sub>f</sub>	7.25 $\pm$ 0.01 <sub>g</sub>
EC (mS cm <sup>-1</sup> )	1.27 $\pm$ 0.01 <sub>g</sub>	1.41 $\pm$ 0.01 <sub>f</sub>	1.65 $\pm$ 0.01 <sub>e</sub>	1.79 $\pm$ 0.01 <sub>d</sub>	2.28 $\pm$ 0.01 <sub>c</sub>	2.50 $\pm$ 0.01 <sub>b</sub>	2.79 $\pm$ 0.01 <sub>a</sub>
TOC (g Kg <sup>-1</sup> )	465.9 $\pm$ 0.99 <sub>a</sub>	420.9 $\pm$ 3.90 <sub>b</sub>	411.2 $\pm$ 2.41 <sub>b</sub>	388.0 $\pm$ 2.27 <sub>c</sub>	378.6 $\pm$ 1.74 <sub>cd</sub>	375.0 $\pm$ 2.25 <sub>cd</sub>	364.8 $\pm$ 3.62 <sub>d</sub>
TKN (g Kg <sup>-1</sup> )	8.06 $\pm$ 0.12 <sub>g</sub>	9.46 $\pm$ 0.20 <sub>f</sub>	10.44 $\pm$ 0.17 <sub>e</sub>	12.13 $\pm$ 0.17 <sub>d</sub>	13.76 $\pm$ 0.17 <sub>c</sub>	15.30 $\pm$ 0.12 <sub>b</sub>	16.84 $\pm$ 0.12 <sub>a</sub>
Total P (g Kg <sup>-1</sup> )	7.81 $\pm$ 0.12 <sub>e</sub>	8.53 $\pm$ 0.04 <sub>e</sub>	9.91 $\pm$ 0.12 <sub>d</sub>	10.44 $\pm$ 0.03 <sub>d</sub>	11.41 $\pm$ 0.08 <sub>c</sub>	13.24 $\pm$ 0.29 <sub>b</sub>	14.44 $\pm$ 0.06 <sub>a</sub>
Total K (g Kg <sup>-1</sup> )	13.27 $\pm$ 0.12 <sub>c</sub>	13.52 $\pm$ 0.13 <sub>c</sub>	13.60 $\pm$ 0.14 <sub>c</sub>	13.75 $\pm$ 0.20 <sub>bc</sub>	14.09 $\pm$ 0.19 <sub>bc</sub>	14.55 $\pm$ 0.05 <sub>b</sub>	16.17 $\pm$ 0.08 <sub>a</sub>
C/N ratio	57.81 $\pm$ 0.95 <sub>a</sub>	44.50 $\pm$ 0.67 <sub>b</sub>	39.39 $\pm$ 0.54 <sub>c</sub>	32.01 $\pm$ 0.40 <sub>d</sub>	27.53 $\pm$ 0.34 <sub>e</sub>	24.51 $\pm$ 0.15 <sub>ef</sub>	21.66 $\pm$ 0.13 <sub>f</sub>
C/P ratio	59.69 $\pm$ 0.83 <sub>a</sub>	49.35 $\pm$ 0.24 <sub>b</sub>	41.51 $\pm$ 0.30 <sub>c</sub>	37.18 $\pm$ 0.25 <sub>d</sub>	33.17 $\pm$ 0.19 <sub>e</sub>	28.35 $\pm$ 0.56 <sub>f</sub>	25.27 $\pm$ 0.27 <sub>g</sub>

Different alphabetical letters in each parameter during vermicomposting represent statistically significant difference at  $p < 0.001$  by Duncan's Multiple Range Test (DMRT)

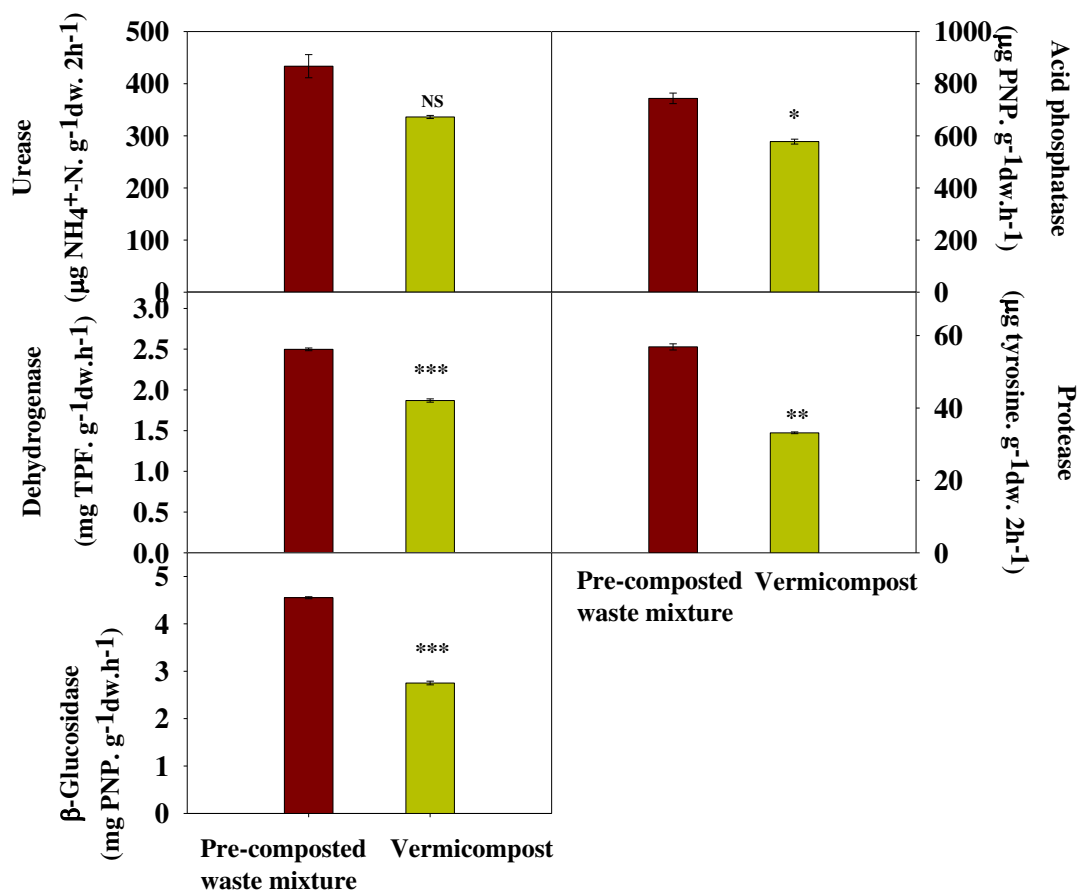
### 3.2. Enzymatic activities

Several enzyme activities were estimated in pre-composted waste mixture and final vermicompost to determine organic matter decomposition during microbial driven vermicomposting process. The variations in the enzyme activities of urease, dehydrogenase, phosphatase, protease and  $\beta$ -glucosidase (main enzymes tangled in urea and aerobic oxidative metabolism, and also breakdown of phosphate esters, polypeptides and polysaccharides, in that order) at two-time intervals are depicted in **Fig. 1**.

The urease activity, which catalyzes hydrolysis of urea ( $\text{CH}_4\text{N}_2\text{O}$ ), yielding  $\text{CO}_2$  and  $\text{NH}_4^+$ , decreased by 22.46% in final vermicompost (t test;  $t=4.14$ ,  $p=0.054$ ) (**Fig. 1**), probably due to elevated levels of  $\text{NH}_4^+\text{-N}$  in the substrate during last phase of the experimental period, which might be responsible for its suppressed activity (Ros et al., 2006; Jurado et al., 2014; Sudkolai and Nourbakhsh, 2017). Also, decreased activity of urease can be ascribed to the reduction of N-substrates, which apparently favor microbial activity (Benitez et al., 1999). Our findings were in accordance to Wu et al., (2017) and Sudkolai and Nourbakhsh (2017), who found similar results for the urease activity. Similarly, the phosphomonoesterases hydrolyzes organic phosphomonoesters to inorganic P (orthophosphate), which inhibits its activity at higher concentration in the substrate (Aira et al., 2007; Fernández-Gómez et al., 2010, 2013). In the present study, acid phosphatase was found to be negatively correlated with available P and a decrease of 22.31% was noticed in final vermicompost (t test;  $t = 6.17$ ,  $p=0.025$ ) (**Fig. 1**). This result is in consistence to Benitez et al., (2005), who noticed an initial increase and subsequent decrease in phosphatase activity while vermicomposting of lignocellulosic olive waste by employing *Eisenia andrei*. The dehydrogenase enzyme plays significant role in biological oxidation of organic compounds and transfers hydrogen from substrates to the acceptor molecules (Page, 1982). Dehydrogenase activity determines overall microbial activity and stabilization of organic substrate during vermicomposting. It was recorded as 2.50 mgTPF.g<sup>-1</sup>dw.h<sup>-1</sup> in pre-composted waste mixture, which was decreased by 25.20% in final vermicompost (t test;  $t=30.30$ ,  $p=0.001$ ) (**Fig. 1**). The decrease of dehydrogenase activity in final vermicompost can be attributed to deprivation and diminution of organic matters, indicating stabilization of the initial waste mixture (Alidadi et al., 2016).

Protease enzyme catalyzes breakdown of proteins to peptides and amino acids (Geisseler and Horwath, 2008; Villar et al., 2016). It's activity results in depolymerization of dissolved organic nitrogen from nitrogenous compounds, which is supposed to be very important in nitrogen cycle, as complex forms are not readily available to the micro-organisms (Schimmel

303 and Bennet, 2004; Aira et al., 2007). The protease activity depends on substrate availability,  
304 therefore, could be useful in analyzing the decomposition rates of organic matter (Aira et al.,  
305 2007). In the present study, protease activity decreased by 41.72% in final vermicompost  
306 (33.15  $\mu\text{g tyrosine. g}^{-1}\text{dw. 2h}^{-1}$ ) as compared to pre-composted waste mixture (56.88  $\mu\text{g}$   
307  $\text{tyrosine. g}^{-1}\text{dw. 2h}^{-1}$ ) (t test;  $t=21.52, p=0.002$ ) (**Fig. 1**). The diminution in protease activity can  
308 be ascribed to low availability of substrate material during terminal phase of the experimental  
309 period, indicating that final product is more stabilized as compared to initial waste mixture.  
310 Similar result was reported by Villar et al. (2016), who confirmed significant reductions in  
311 protease activity during progression of vermicomposting experiment with sewage sludge.  
312 Likewise,  $\beta$ -glucosidase is involved in carbon cycle, which catalyzes the breakdown of  
313 glucosides to glucose and therefore associated with the turnover of carbon (Sinsabaugh and  
314 Moorhead,1994; Alef and Nannipieri, 1995). Its activity can be induced by the substrate  
315 therefore depends on substrate availability (Alef and Nannipieri, 1995). During  
316 vermicomposting process,  $\beta$ -glucosidase is synthesized and released presumably due to the  
317 breakdown of organic wastes and/or increase in microbial growth and activation of their  
318 metabolism (Eivazi and Tabatabai, 1990; Aira et al., 2007). In this study,  $\beta$ -glucosidase activity  
319 declined by 39.56% in final vermicompost and was found to be 2.75  $\text{mg PNP. g}^{-1}\text{dw.h}^{-1}$  as  
320 compared to 4.55  $\text{mg PNP. g}^{-1}\text{dw.h}^{-1}$  in pre-composted waste mixture (t test;  $t=34.08, p=0.001$ )  
321 (**Fig. 1**). The decline in  $\beta$ -glucosidase activity can be credited to depletion of organic substrate  
322 in older layers (Aira et al., 2007). The substantial presence and maintenance of extracellular  
323 enzyme activities during end phase of experimental period could be attributed to the  
324 development of complexes between extracellular enzymes and humic substances that gets  
325 protected from denaturation and degradation processes (Jurado et al., 2014; Villar et al., 2016).  
326  
327



Level of statistically important difference at  $p < 0.05$  (\*),  $p < 0.01$  (\*\*),  $p < 0.001$  (\*\*\*) and NS (non- significant) by Paired Student's t-test

**Fig. 1.** Changes in enzymatic activities of pre-composted waste mixture and final vermicompost

### 3.3. Fourier transform infrared analysis

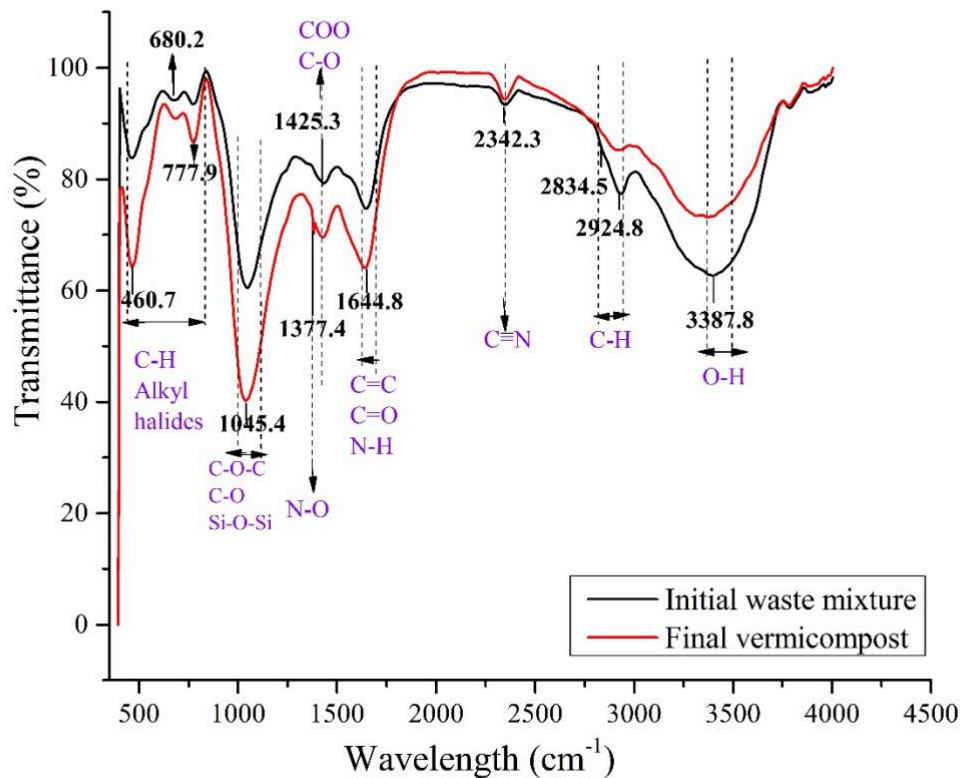
The fourier transform infrared (FT-IR) spectroscopy facilitates identification of chemical functional groups of a matter where the absorbance intensity change is used to assess vermicompost stability (Lim and Wu, 2015). In **Fig. 2**, the relevant spectral bands of initial waste mixture and final vermicompost are presented. The assignment of infrared (IR) absorptions ascribed to specific functional groups is summarized in **Appendix S2**.

In comparison to initial waste mixture, the FT-IR spectra of final vermicompost presented significant lessening in the peak intensity at  $3387.8\text{ cm}^{-1}$  reflecting decomposition of phenols and carbohydrates due to reduction in OH and  $\text{CH}_2$  structures. Lim and Wu (2015), found that

decreased peak intensity at 3328  $\text{cm}^{-1}$  showed degradation of carbohydrates in palm oil mill effluent (POME) vermicompost. Likewise, Hussain et al. (2015) stated significant reduction in the peak intensity at 3448  $\text{cm}^{-1}$  depicting reduction in phenolic content. Gupta and Garg (2009) reported reduced band height at around 3100-3600  $\text{cm}^{-1}$  in the cow dung vermicompost in comparison to initial substrate. The reduction in peak intensity at 2924.8 and 2834.5  $\text{cm}^{-1}$  in final vermicompost suggested degradation of lipid and carbohydrates due to decrease in aliphatic structures. Droussi et al. (2009), observed decrease of methylene bands (C-H stretch) at 2920 and 2850  $\text{cm}^{-1}$  during progression of composting of olive-mill residues. Comparable outcomes were noticed by Gupta and Garg (2009) and Makni et al. (2010) while maturation study of organic waste compost. No obvious change of intensity was noticed in  $\text{C}\equiv\text{N}$  stretching vibrations of nitriles at 2342.3  $\text{cm}^{-1}$ . Soobhany et al. (2017), observed  $\text{C}\equiv\text{N}$  vibrations of nitriles at 2329  $\text{cm}^{-1}$  in compost and vermicompost of paper waste. A prominent increase in the band intensity at 1644.8  $\text{cm}^{-1}$  was noticed in final vermicompost as compared to initial waste mixture. This change could have been attributed to increase in aromatic groups during vermicomposting. Droussi et al. (2009) found increase in band intensity at 1650-1640  $\text{cm}^{-1}$  due to aromatic  $\text{C}=\text{C}$  stretching in compost produced from olive mill residues. Similar result was observed by Makni et al. (2010) whilst studying maturity of organic waste composts. Soobhany et al. (2017) observed prominent shoulder around 1635 and 1630  $\text{cm}^{-1}$  due to N-H bending vibrations correspond to Amide I species and  $\text{C}=\text{C}$  stretching of aromatic compounds in compost and vermicompost samples. No remarkable change was found at peak intensity of 1425.3  $\text{cm}^{-1}$ , followed by emergence of a sharp peak at 1377.4  $\text{cm}^{-1}$  due to N-O stretching of nitro group (Smidt et al. 2002; Hussain et al. 2015). Emergence of similar N-O stretching vibration was noticed by Hussain et al., (2015) and Rajiv et al. (2013) at peak intensity of 1384 and 1381  $\text{cm}^{-1}$  respectively in vermicompost samples. The peak intensity increased at 1045.35  $\text{cm}^{-1}$  due to elevated levels of aromatic ethers and polysaccharides. Droussi et al. (2009) observed large peak bandwidth around 1070-1030  $\text{cm}^{-1}$  due to aromatic ethers and carbohydrates in compost. Analogous results were described by Hussain et al. (2015) and Rajiv et al. (2013). An appreciable increase in peak intensities at 777.9, 680.2 and 460.7  $\text{cm}^{-1}$  was observed in final vermicompost (**Fig. 2**). This result was in accordance to Makni et al. (2010), who noticed prominent increase in peak intensities at 873.4, 778.5 and 467.9  $\text{cm}^{-1}$  due to increase of silicates (Si-O-Si), aromatic ethers and polysaccharides (C-H stretch) in ready compost samples. The peaks between 777.9 and 460.7  $\text{cm}^{-1}$  with minor variations in final vermicompost can equally be ascribed to alkyl halides and polysulfide groups (Reusch, 2013). This finding was in consistent with Soobhany et al. (2017), who observed small differing peaks

between 875 and 456  $\text{cm}^{-1}$  due to alkyl halides and polysulfide groups in compost and vermicompost and were more intense in vermicompost samples.

The findings of FT-IR study revealed disappearance of polysaccharides, carbohydrates and aliphatic methylene from the initial waste mixture whilst aromatic compounds, nitro group and humic structures appeared in MSW vermicompost depicting its stability and maturity.



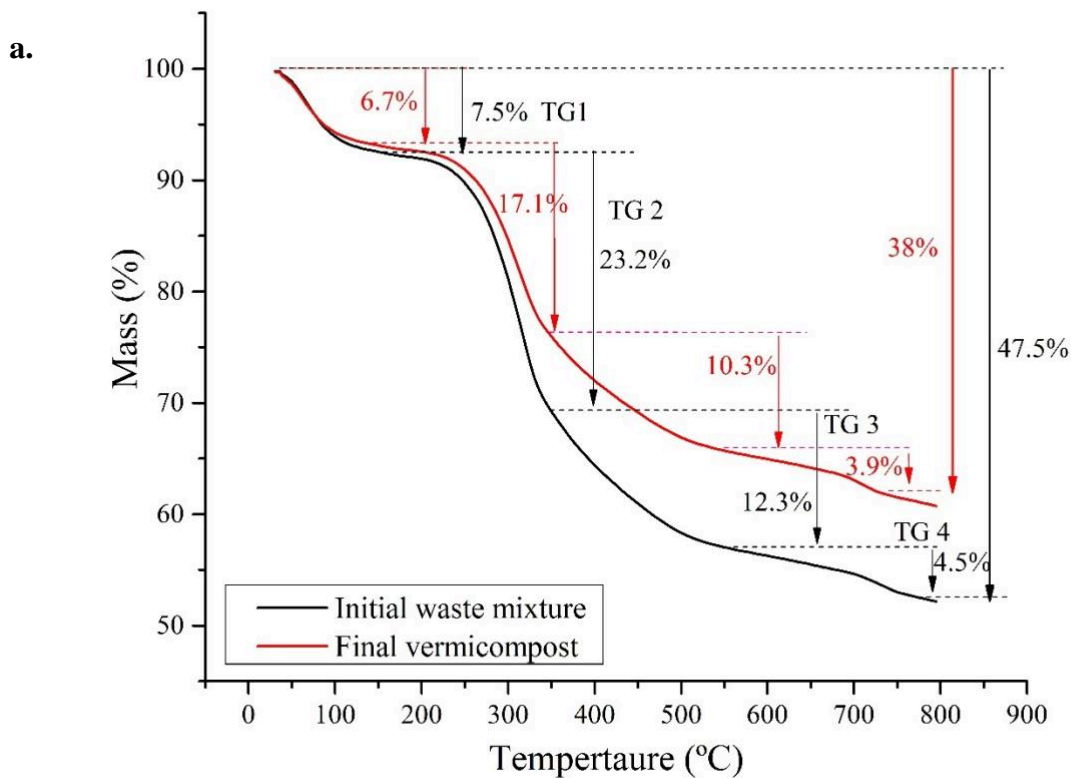
**Fig. 2.** FT-IR spectra of the pre-composted waste mixture and final vermicompost

### 3.4. Thermogravimetric analysis

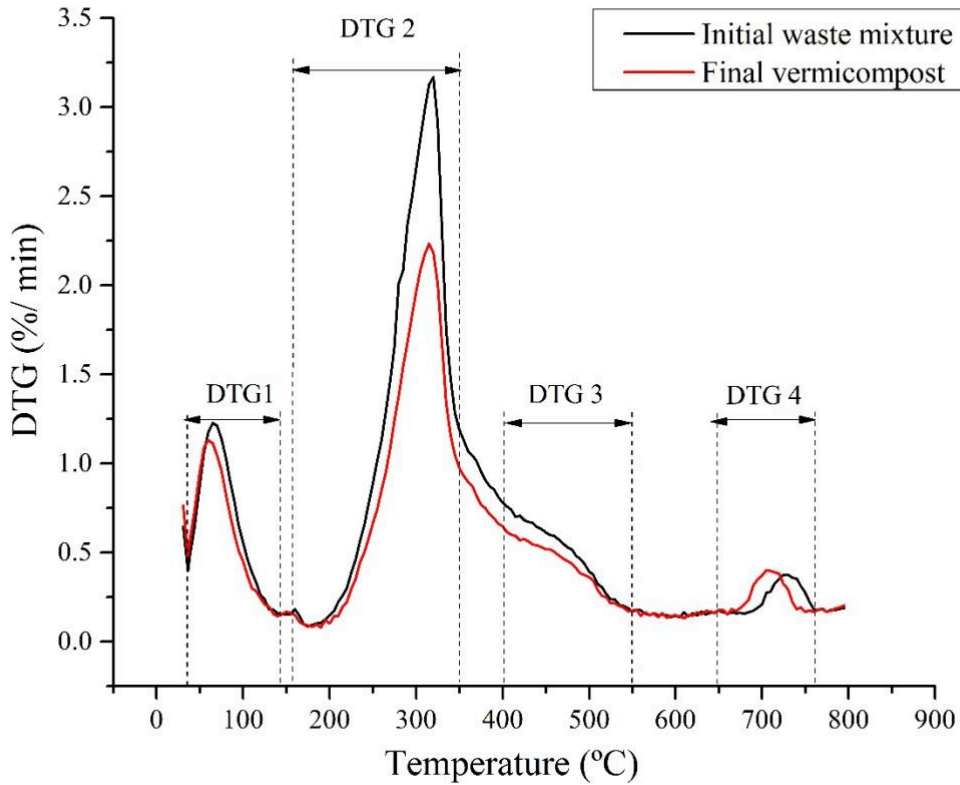
Thermogravimetry (TG) is a thermal technique which is used to examine the changes in mass of a sample at increasing temperature and constant heating rate. TG analysis of substrate material at two-time intervals during vermicomposting revealed thermal stability of samples explained by mass loss due to dehydration, oxidation and degradation (Bhat et al., 2017; Khatua et al., 2018). The derivative thermogravimetric (DTG) curves were found from the first derivative of thermogravimetric profiles. The TG and DTG curves of the initial waste mixture and final vermicompost are presented in **Fig. 3 a & b**. Both the samples showed gradual decrease in mass under heating conditions. TG profiles showed higher percentage of mass loss in the initial waste mixture as compared to final vermicompost (**Fig. 3a**). Under atmospheric conditions, the total mass losses at 800 °C were 47.5 and 38% for initial waste and vermicompost, respectively (**Fig. 3a**). These changes in mass percentage could be ascribed to higher volatile solids in initial waste mixture (Lim et al., 2014). Consequently, vermicompost was comparatively more stabilized as it contained higher amount of heat resistant compounds due to increased molar complexity of carbohydrates and extent of aromaticity (Ravindran et al., 2013; Kahtua et al., 2018). The decrease in mass percentage up to 140 °C was owed to the dehydration or loss of residual water from the specimens that could have been seen in the first peak of DTG profile in the temperature ranged between 30-140 °C (DTG1) (**Fig. 3b**). Kahtua et al., (2018) and Soobhany et al., (2017) observed analogous results in the vermicompost samples of banana stem and MSW respectively. Following dehydration, a progressive decrease in mass percentage was noticed at ~155-350 °C (DTG2). This peak was ascribed to the decarboxylation reactions of easily degradable materials and thermal decomposition of carbonaceous biomass such as aliphatic compounds, carbohydrates, and carboxylic groups (Ravindran et al., 2013; Soobhany et al., 2017; Kahtua et al., 2018). The decay of cellulose, hemicellulose and microbial cell wall could have also been responsible for the appearance of this peak (Kaloustian et al., 1997; Lim and Wu 2015). The peak intensity of DTG2 declined significantly during the vermicomposting progression. The reduction in peak intensity can be ascribed to biochemical conversion of readily decomposable organic matter into more complex humic like substances (Gómez et al., 2007; Lim and Wu, 2015; Wu et al., 2011). The decrease in intensity was in confirmation with the FT-IR study (**Fig. 2**). A peak (DTG3) appeared at higher temperature range of ~400-550 °C was ascribed to the mass loss owing to the thermal dissociation and breakdown of extra stable and complex aromatic structures such as lignin (El Ouaquoudi et al., 2015; Soobhany et al., 2017; Kahtua et al., 2018). Thermal degradation of



aliphatic substances including long chain hydrocarbons and nitrogenous compounds was also supposed to be liable for mass loss above  $\sim 400$  °C (Ravindran et al., 2013). The peak DTG3 decreased and moved slightly towards higher temperature and found at 474 °C in final vermicompost as equated to 468 °C in initial waste mixture. The shift in peak towards higher temperature showed growth in molecular complexity and aromaticity in vermicompost depicting maturity and stability of sample (Smidt and Lechner, 2005). Eventually, the peak (DTG4) emerged at the temperature range of 650-750 °C was credited to thermal decay of carbonates (Wu et al., 2011) (**Fig. 3b**). Its intensity increased slightly with progression of vermicomposting depicting mineralization of organic matter.



b.

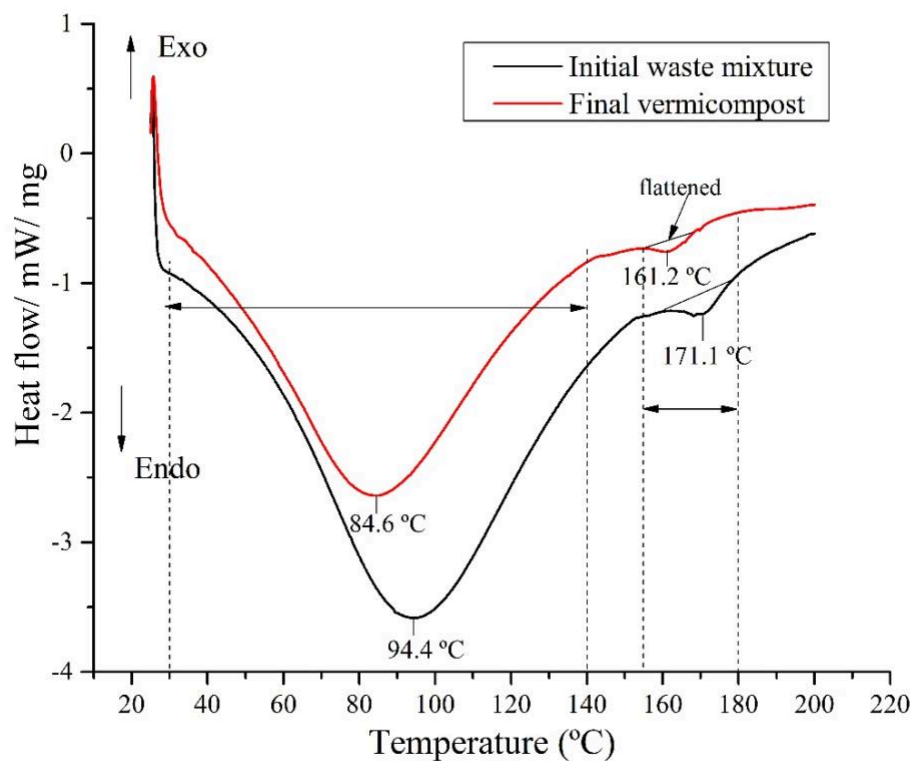


**Fig. 3. a.** Percentage mass loss and **b.** derivative thermogravimetry (DTG) curves from thermogravimetry exploration of the pre-composted waste mixture and final vermicompost

### 3.5. Differential scanning calorimetry

Differential scanning calorimetry (DSC) is a thermo-analytical procedure which is used to assess the physico-chemical changes during the composting and vermicomposting which exhibit exothermic reactions under heat capacity. The DSC curves of initial and final vermicompost are revealed in **Fig. 4**. DSC curve of initial feed mixture showed two energy requiring endothermic peaks at 94.4 °C and 171.1 °C respectively. These peaks were characterized by loss of residual water and peripheral polysaccharide chains (Crittter and Airoldi, 2006; Soobhany et al., 2017). Analogous results were conveyed by Soobhany et al. (2017), who observed two endothermic peaks at 83.8 and 166.4°C. In the present study, a sharp low temperature endothermic peak was detected at 84.6 °C, shows higher maturity of vermicompost sample as compared to initial waste mixture (Provenzano et al., 2000; Soobhany et al., 2017) (**Fig. 4**). Likewise, second peak at 161.2 °C was in accordance to DTG2 (**Fig. 3b**) that can be attributed to the energy needed for bond breakage during degeneration of aliphatic

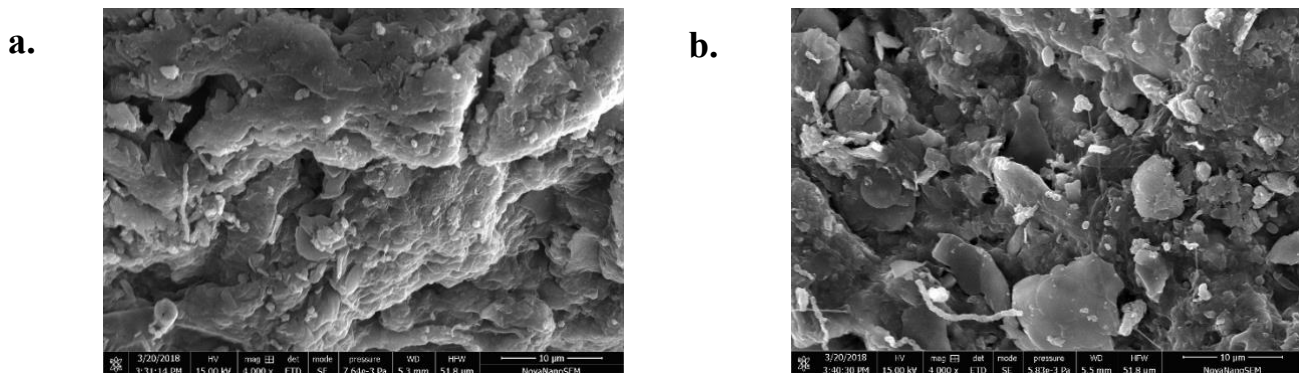
and carbohydrate compounds. Furthermore, molecular repositioning and orientation due to degradation of aliphatic, carbohydrates, peptides and other aromatic compounds might have also been responsible for the appearance of second peak (Fernández et al., 2012; Soobhany et al., 2017). The second endothermic peak at 161.2 °C appeared slightly flattened in final vermicompost due to reduced degradation of complex compounds (present in lesser amount) depicting stabilization and maturity of final vermicompost. This result was in accordance to Soobhany et al. (2017), who found virtually flattened endothermic peak at ~165°C in vermicompost sample as compared to compost. The DSC curves showed that vermicompost was more stabilized product as compared to the initial waste mixture depicted by reduced intensity of endothermic peaks up to 220 °C suggesting low availability of complex compounds in vermicompost sample.



**Fig. 4.** Differential calorimetry curve of the initial feed mixture and final vermicompost

### 3.6. Scanning electron microscopy (SEM)

Snapshots obtained from SEM of initial waste mixture and final vermicompost are compared in **Fig. 5**. The study discovered that initial waste mixture was relatively compacted and flock like structure, whereas the vermicompost had more porous, fragmented and granular structure (**Fig. 5a & b**). During vermicomposting, the earthworms (*Eisenia fetida*) ingested and pulverized the initial waste mixture in the gizzard (Ali et al., 2015). Further disintegration of substrate material was assisted by different enzymes and enteric microbial activities mainly by hydrolytic bacteria present in the earthworm's gut (Arancon et al., 2005; Bhat et al., 2017). Our results are in consistence with Unuofin and Mnkeni (2014), who observed fine granular structure of vermicomposts produced from cow dung and paper waste mixtures. Similar results were reported by various researchers who performed SEM exploration to elucidate changes in surface morphology of initial substrate and final vermicomposted mixtures (Kumar et al., 2014; Rajpal et al., 2014; Bhat et al., 2015; Hussain et al., 2016).



**Fig. 5.** Scanning electron microscopy (4000x) of **a.** pre-composted waste mixture and; **b.** final vermicompost

## 4. Conclusion

The OPMSW mixed with CD could be utilized in vermicomposting employing earthworm spp. *Eisenia fetida*. The enrichment of TKN, TP and TK contents, and decrease in TOC, C/N and C/P ratios depicted maturity and stability of the final product. Likewise, decline of urease, phosphatase, protease and  $\beta$ -glucosidase activities suggested decomposition of N-substrates, phosphate esters, polypeptides and polysaccharides, respectively in final vermicompost. The spectroscopic and thermal analyses further supported stability and maturity of the end product reflected by reductions in readily degradable polysaccharides and aliphatic compounds and increase in aromatic groups. Also, SEM micrographs revealed relatively larger surface areas

and much flakier structure of final vermicompost. In conclusion, these findings will contribute to better understanding of vermicompost stability and maturation study using different conventional and advanced instrumentation. The spectroscopic, thermal and structural analyses could easily be used to establish maturity and stability in compost science, which are comparatively less time consuming and more accurate over conventional analyses. The vermistabilization of OPMSW could also help to develop an eco-friendly and alternative approach for nutrient recovery and waste management for a green and healthy environment.

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**Conflict of Interest** On behalf of all authors, the corresponding author states that there is no conflict of interest.

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