

REVIEW

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# Instrumental characterization of organic wastes for evaluation of vermicompost maturity

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## Abstract

Instrumental analysis of vermicompost with the help of modern technologies provides essential information on its maturity, before it can be used for agricultural application. Nowadays, vermicompost is considered as a promising organic alternative to chemical fertilizers in agriculture and horticulture. The objectives of this review are to summarize the sophisticated instrumental techniques such as scanning electron microscopy (SEM), fourier transform infrared (FT-IR) spectroscopy, thermogravimetry (TG), ultraviolet-visible (UV-vis) spectroscopy techniques, physico-chemical parameters (pH, electrical conductivity, organic carbon content, C:N ratio, nitrogen, phosphorus, potassium, sodium, calcium) and biological indicator (germination index) to determine the maturity of vermicompost produced from organic wastes. These techniques are reliable, fast and are capable of tracking organic waste degradation during the bioconversion process and fertilizing ability of the final product. SEM analysis provides essential information on surface morphology of vermicompost samples. The SEM micrograph of final vermicompost reveals disaggregation. In contrast, the initial SEM micrograph reflects robust and relatively contiguous structures. FT-IR spectroscopy technique is used to confirm the decomposition of polypeptides, polysaccharides, aliphatic, aromatic, carboxylic, phenolic groups and lignin during vermicomposting of organic wastes. TG method is used to characterize organic waste mineralization where progressive reduction in the mass loss of vermicompost indicates net mineralization and degradation. UV-vis spectroscopy is used to assess the degree of humification. The sharp fall in humification index during vermicomposting process indicates high level of organic material humification. Changes in physico-chemical and biological parameters are also an indicative parameter for organic waste mineralization as well as vermicompost stability and maturity.

**Keywords:** Earthworms, Vermicompost, Maturity, Instrumental techniques

## Review

### Introduction

Vermicomposting is a biological process used to convert complex organic wastes into a stabilized nutrient-rich fertilizer called vermicompost. Vermicomposts are peat-like materials containing plant growth hormones and nutrients in plant available forms (Lim et al. 2016). In vermicomposting process, earthworms and microbes of their gut degrades organic waste and forms a stable and mature product (Bhat et al. 2013; Lim et al. 2016). Organic waste recycling and its use as a soil conditioner is the

attractive method for waste management. The application of organic manures produced from organic wastes by vermicomposting is one of the ways to maintain soil fertility and reduce the soil pollution (Ostos et al. 2008; Bhat et al. 2016a). Determination of vermicompost maturity is important before it can be used with agricultural applications. The maturity of vermicompost process involves changes in texture and structure which can be determined through scanning electron microscopy (SEM), fourier transform infrared (FT-IR) spectroscopy, thermogravimetry (TG) and ultraviolet-visible (UV-vis) spectroscopy techniques (Ravindran et al. 2013; Lim and Wu 2016). SEM analysis provides essential information on surface morphology and numerous surface irregularities in the vermicomposted mixtures that confirms the

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maturity and degradation of waste by earthworms (Bhat et al. 2014, 2015a). The final vermicomposted mixtures revealed a more fragmented texture than initial waste mixtures. FT-IR spectroscopy technique is used to confirm the decomposition of several functional groups. In FT-IR spectrum, the presence or absence of several absorbance bands in the functional groups indicates the mineralization process (Gupta and Garg 2009). In FT-IR spectroscopy, the evaluation process is based on changes in the relative intensity of bands (Lim and Wu 2016) and thus can be a reliable technique for evaluation of its maturity. TG analysis is used to characterize mineralization of organic wastes. The TG curves provide complete information on loss of mass, mineralization and decomposition of organic waste under different temperatures (Ravindran et al. 2013). UV-vis spectroscopy determines the maturity of any substrate by assessing the rate of humification. Sharp reduction in the humification index and degradation of complex aromatic compounds confirms the maturity of the vermicompost (Lim and Wu 2015). These techniques are reliable, fast and are capable of tracking organic waste degradation during the vermicomposting process. During the process of vermicomposting, physico-chemical (pH, electrical conductivity, organic carbon content, C:N ratio, nitrogen, phosphorus, potassium, sodium, calcium) and biological parameters (germination index) also depicts biochemical changes in the organic wastes and shows rapid mineralization and decomposition rate of the final feedstock (Majlessi et al. 2012; Bhat et al. 2015a, 2016b, c). The land-applied immature compost/

vermicompost can give rise to a serious N-deficiency in crops (Chikae et al. 2006; Komilis and Tziouvaras 2009). Addition of immature compost/vermicompost can inhibit the plant growth by the production of phytotoxic substances (Gomez-Brandon et al. 2008). Thus, the present review article will focus on instrumental techniques such as SEM, FT-IR, TG, UV-vis spectroscopy and physico-chemical parameters (pH, electrical conductivity, organic carbon content, C:N ratio, nitrogen, phosphorus, potassium, sodium, calcium) used to determine the maturity of vermicompost and bio-transforming wastes into manure. Use of instrumental techniques, its procedure, advantages and limitations are discussed here.

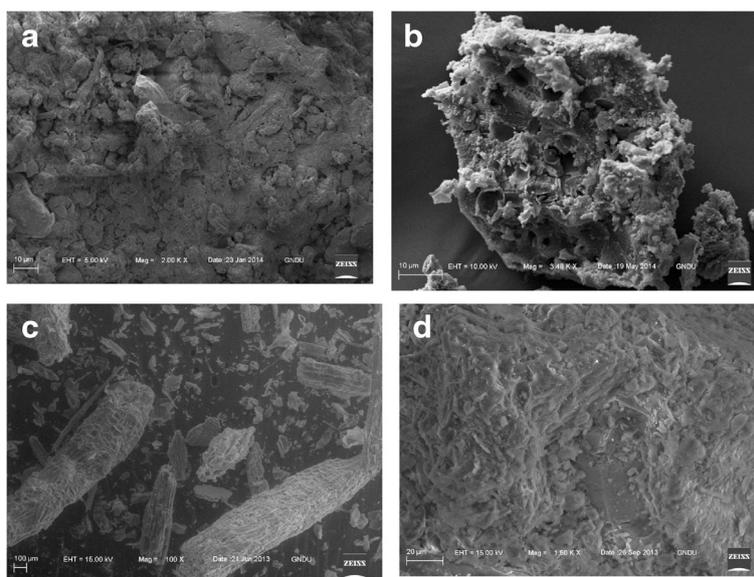
### Sample analysis procedure

#### SEM analysis

SEM is a powerful magnification technique that utilizes focused beams of electrons to obtain surface information. SEM analysis provides essential information on surface morphology of vermicompost and control samples. Earthworms grind the feed mixtures, and further action by microbes and enzymes in the earthworm gut disaggregates the substrates. SEM micrographs (Fig. 1) of final vermicomposted mixtures reveal the presence of greater number of surface changes, which indicates biodegradation and mineralization of waste by the earthworms (Bhat et al. 2015a).

#### Procedure

The initial and final dried vermicomposted samples are fixed on a circular metallic sample holder by using



**Fig. 1** Scanning electron microscopy. **a** Pre-vermicomposted cattle dung. **b** Post-vermicomposted cattle dung. **c** Pre-vermicomposted bagasse. **d** Post-vermicomposted bagasse

double-sided adhesive tape and then the samples are coated with gold using sputter coater for clear visibility of picture, followed by micrographs of surface texture at different magnifications of SEM (Bhat et al. 2015a, b).

#### Advantages and limitations

The high-resolution micrographs produced by SEM provide morphological, topographical and compositional information of any material that makes them invaluable in a variety of science and technology applications. SEM technique can examine and evaluate surface fractures, microstructures, surface contaminations and spatial variations in chemical compositions and can identify crystal-line structures. It is an important technique for the judgement of the physical changes in the texture during the vermicomposting process. SEM micrographs are found to be very significant and economic for the evaluation of vermicompost maturity and stability. It can also measure surface area of the vermicompost.

SEM technique is expensive and must be free of any possible magnetic or electric interference. SEM requires special training to operate as well as sample preparation. In addition, SEM is only limited to solid and inorganic samples.

#### FT-IR spectroscopy analysis

FT-IR spectroscopy technique is used to confirm the decomposition of polypeptides, polysaccharides, aliphatic, aromatic, carboxylic, phenolic groups and lignin during vermicomposting process. FT-IR spectrum of initial and final vermicomposted samples are generally obtained in the mid-infrared area range of 4000–400  $\text{cm}^{-1}$ . It is one of the most reliable techniques for determination of vermicompost maturity. The different peaks/values show the presence or decomposition of their group in the sample. The FT-IR interpretation of numerous studies is shown in Table 1.

#### Procedure

About 1 mg sample with 100 mg potassium bromide (KBr) are mixed and pressed into a pellet at a pressure of 1 MPa (megapascal). The pellets are about 1 mm in thickness and 10 mm in diameter. The different bands are analyzed to quantify the bioremediation process (Ravindran et al. 2013; Lim and Wu 2016).

#### Advantages and limitations

FT-IR spectroscopy is used to study compost/vermicompost without any chemical treatment likely to cause unsuitable reactions. This technique is simple, quick, highly sensitive and informative for evaluation of vermicompost maturity and stability. Solids, liquids as well as gas can be analyzed with FT-IR spectroscopy and is environment friendly as it produces remarkably less waste.

**Table 1** Assigned values for different peaks in FT-IR

Wave number/ $\text{cm}^{-1}$	Vibration	Functional group
3300-3500	O-H stretch	Phenols and carboxylic groups
3180-3090	NH <sub>2</sub> stretch	Primary amides
2925-2850	C-H stretch	Aliphatic methylene groups of lipids and fats
2590-2560	S-H stretch	Thiol group
1740-1720	C=O	Aldehyde, ketone, carboxylic acids and esters
1655-1640	C=O C=C	Amide I, carboxylates Aromatic ring modes, alkenes
1570-1550	N-H deformation and C=N stretch	Amides II
1515-1510	Aromatic C=C stretch	Lignin
1421-1410	COO <sup>-</sup> stretch C-N stretch	Carboxylic acids Amide III
1384	N-O stretch	Nitrate band
1320	C-N stretch	Aromatic primary and secondary amines
1000-1100	C-O stretch Si-O stretch	Polysaccharides Clay minerals
875	C-O out of plane	Carbonate
850-750	NH <sub>2</sub> out of plane	Primary amine group
750-700	N-H wag	Secondary amine group
680-610	S-O bends	Inorganic sulphates

Source: Grube et al. (2006); Smidt and Meissl (2007); Deka et al. (2011); Lv et al. (2013); Ravindran et al. (2013)

The disappearance or the appearance of new bands in the FT-IR spectroscopy provides essential information about the organic matter evolution and its interaction with heavy metals present in wastes. FT-IR spectroscopy can easily identify organic and inorganic compounds.

FT-IR spectroscopy is a single beam technique. It cannot detect atoms or monoatomic ions. It is impossible to measure the level of an element in a substance, unless it is present as a part of a molecule whose spectrum can be detected.

#### TG analysis

TG is a technique in which sample weight is measured as a function of temperature (Plante et al. 2009). It provides essential information on loss of mass, mineralization and decomposition of organic waste under different temperatures. The loss of mass is measured between the inflection points of the TG curve (Karathanasis and Harris 1994). The progressive reduction in the mass loss during the process of vermicomposting indicates net mineralization and degradation. The final vermicomposts have lower mass loss as compared to

the pre-vermicompost waste which indicates higher maturity of vermicompost.

#### **Procedure**

About 50 mg sample is crushed in an agate mortar. The samples are then sieved to  $\leq 2$ -mm pore size and analyzed for TG analysis. TG analysis is generally performed in the temperature range of 30 to 1200 °C under heating rate of 10 °C/min. The pressure is always maintained at 101 kPa (kilopascal) (Ravindran et al. 2008, 2013).

#### **Advantages and limitations**

The main use of TG analysis includes measurement of a compost/vermicompost's thermal stability and composition. We can evaluate the degree of maturation and stabilization of compost/vermicompost without extraction and fragmentation procedures to isolate humic-like substances. This technique is quite inexpensive, rapid, information rich, require little sample preparation and can give reproducible results.

TG analysis requires knowledge of the sample to relate loss of weight to decompositions.

#### **UV-vis spectroscopy analysis**

In vermicomposting, UV-vis spectroscopy analysis is used to assess the degree of humification which is often called as humification index ( $Q_{4/6}$ ). Humification is the decomposition and breakdown of organic material. Humification index value of less than 5 indicates higher humification of organic material (Ravindran et al. 2013). A lower value of humification index reflects a high level of aromatic condensation which indicates a high degree of organic matter humification (transformation of organic matter into humus).

#### **Procedure**

The degree of humification in initial and final vermicompost is determined by the method of Zbytniewski and Buszewski (2005). One gram of initial and final vermicompost are taken separately in a 250-mL polyethylene flask and extracted with 50 mL of 0.5 M NaOH by shaking for 2 h. The contents of each flask were left undisturbed overnight, and the suspension was centrifuged at 3000 rpm for 25 min. The absorbance (A) of each supernatant is measured at 472 nm ( $A_{472}$ ) and 664 nm ( $A_{664}$ ). The degree of humification is calculated by the absorbance ratio  $Q_{4/6} = A_{472}/A_{664}$ .

#### **Advantages and limitations**

UV-vis Spectroscopy is fast, simple and inexpensive method to determine the degree of humification during the vermicomposting process. The absorption spectra of alkali-soluble humic substances are widely used for maturity determination in final vermicompost. The concentration of

aromatic compounds generally increases during the humification process.

UV-vis spectroscopy technique requires frequent calibrations to retain the accuracy and precision of the instrument.

#### **Physico-chemical analysis**

About 25 g of each sample is kept to perform different physico-chemical analysis before and after vermicomposting. The samples are air dried and crushed to fine powder by a grinder and stored in airtight plastic packets. The powdered samples are subjected to physico-chemical parameters as follows.

#### **pH and electrical conductivity (EC)**

A suspension is made in a flask by mixing sample with double distilled water (1:10 w/v). The suspension is shaken over a shaker for 40 min. The supernatant are taken out and analyzed for pH and EC. The pH meter and EC meter should be calibrated prior to its use. Vermicomposts of pH 6–8.5 and EC value of below 4.0 mScm<sup>-1</sup> are excellent for agricultural soils.

#### **Organic carbon (OC)**

Dry combustion method of Nelson and Sommers (1982) is used for determining the OC. A weight of 500 mg of air-dried sample (<2 mm) was transferred into the pre-weighed china crucible. The samples are ignited in a muffle furnace at 550 °C for 60 min. The furnace is allowed to cool, and the ash produced is weighed again. OC is calculated from the below given formula.

$$\text{Ash \%} = \frac{\text{Weight of sample left after ignition (ash)}}{\text{Weight of sample taken}} \times 100$$

$$\text{Organic carbon percentage (\%)} = \frac{100 - \text{ash percentage}}{1.724}$$

where 1.724 is the conversion factor

#### **Wakley-Black titration method for organic carbon**

This method involves a wet combustion of organic matter with a mixture of potassium dichromate and sulphuric acid at about 125 °C. To compensate for incomplete destruction, an empirical factor 1.3 (correction factor) is applied in calculation of the result (Walkley and Black 1934).

Procedure: In 1 g of sample, add 10 ml of dichromate solution and 20 ml concentrated sulphuric acid and allow to stand for 30 min. Add 250 ml distilled water and 10 ml of phosphoric acid and allow to cool. Add 1 ml indicator and titrate with ferrous sulphate solution. The colour changes to purple and end result with green.

Calculations:

$$\text{OC}(\%) = M \times \frac{V_1 - V_2}{S} \times 0.39 \times \text{mcf}$$

M Molarity of ferrous sulphate solution

$V_1$  milliliter of ferrous sulphate solution required for blank

$V_2$  milliliter of ferrous sulphate solution required for sample

S weight of sample taken

0.39  $3 \times 10^{-3} \times 100\% \times 1.3$  (where 3 is equivalent weight of carbon, 1.3 is compensation factor)

Mcf moisture correction factor

After this, you can also calculate

$$\% \text{ Organic Matter} = 2 \times \% \text{ OC}$$

### Nitrogen (N)

N is determined by micro-Kjeldhal method (Bremner and Mulvaney 1982).

Air dried sample (0.5 g) is taken into 100-ml digestion flask along with 10 ml of digestion acid mixture (1 g digestion mixture ( $\text{K}_2\text{SO}_4$ :  $\text{CuSO}_4$  and  $\text{SeO}_2$  in the ratio of 10:4:1) in 10 ml concentrated sulphuric acid). Initially (about 30 min), this flask is heated at low temperature until the contents became light yellow green. The digest is cooled and the final volume is made 50 ml with distilled water. An aliquot of 10 ml is taken from the digested sample and reacted in micro Kjeldhal apparatus with 10 ml of 40% NaOH to produce ammonia. Boric acid indicator (5 ml) is taken into a flask and placed below the condenser of Kjeldhal apparatus so that the tip of the condenser dips into the boric acid indicator to absorb liberated ammonia.

The condensate is titrated with 0.01 N HCl using a burette. The colour change at end point is from greenish-blue to permanent light pink.

Calculation

$$\% N = \frac{(a-b) \times N \times 1.4}{S}$$

Where

a sample titrate (ml)

b blank titrate (ml)

N Normality of HCl used

1.4 multiplication factor

S weight of sample (g)

### Phosphorus (P)

P is determined by the method of John (1970).

0.5 g of dry material is taken in 100-ml digestion flask along with 15 ml of diacid mixture (nitric acid and perchloric acid 4:1). The mixture is digested on hot plate till it becomes colourless. Then, contents of the sample are diluted to about 30 ml with distilled water, filtered and

transferred to 50-ml flask and total volume is made 50 ml by double distilled water. From each flask, 1 ml of aliquot is taken in 50-ml flask and 5 ml of mixed reagent (1.5 g of ascorbic acid is added to 100 ml of stock solution) is added. Total volume is made to 50 ml by double distilled water. After 30 min, the absorbance of solution is measured at 882 nm using a spectrophotometer.

Calculation

$$\begin{aligned} \text{P concentration} &= \text{Absorbance} \times \text{Graph factor} \\ &= (A \text{ mg/l}) \end{aligned}$$

To convert it into percent,  $P \text{ (as } \text{PO}_4^{3-}) = (3A/100) \%$

### Potassium (K), sodium (Na) and calcium (Ca)

K, Na and Ca are measured flame photometrically in the diacid digest of the samples. The samples are digested and prepared as given in Phosphorus method. Standard stock solution of 1000 mg/l of K, Na and Ca are prepared by dissolving 0.191 g of KCl, 0.2543 g of NaCl in 100 ml distilled water and 2.497 g of  $\text{CaCO}_3$  and 10 ml of concentrated HCl in 300 ml of distilled water. By making use of this stock solution, standard solutions of 20, 40, 60, 80 and 100 mg/l are prepared. The concentrations of Na and K, in sample solutions, are determined by readings obtained from flame photometer using a standard curve (graph factor).

### Biological analysis

#### Seed germination index

Germination index (GI) is the direct indicator of vermicompost maturity as it directly tests whether the amount of final vermicompost can enhance growth of plants or not. The effect of vermicompost maturity on seed germination index is usually determined with different plant seeds, using the method described by Zucconi et al. (1981). Fresh extract of vermicompost and distilled water (1:1) are poured into a petri dish lined with filter paper. The seeds are placed on the filter paper and then incubated at 25 °C under dark cover for 5 days. The number and root length of germinated seeds are recorded. The GI is determined by the formula:

$$\text{GI} = \frac{\text{Seed germination} \times \text{root length of the treatment}}{\text{seed germination} \times \text{root length of the control}} \times 100$$

It is a comparative test of knowing the maturity of pre- and post-vermicomposted samples.

### Relevance and use of physico-chemical parameters for the evaluation of vermicompost maturity and stability

#### pH and electrical conductivity

The pH and EC are important parameters for the evaluation of vermicompost maturity and quality. pH usually decreases from alkaline to neutral, during the process of

vermicomposting (Suthar 2010). The change in pH towards acidic or neutrality may be due to the formation of organic acids and mineralization of organic waste which leads to the production of both the ammonium ions and humic acids (Komilis and Ham 2006). According to Ndegwa and Thompson (2000), the changes in pH of final vermicompost are due to decomposition of organic waste into organic acids. Hogg et al. (2002) reported that the vermicomposts possessing pH ranges of 6–8.5 are excellent for application of soil. EC measures the amount of salinity in an organic material and is a good indicator of vermicompost quality used in agriculture (Lim et al. 2014). According to Shak et al. (2014), the decrease in EC during vermicomposting may be due to the precipitation or leaching of soluble salts and mineralization of organic acids. Lasaridi et al. (2006) observed that the plants have a maximum EC tolerance limit of  $4.0 \text{ mScm}^{-1}$  and below this value of EC, manure can be applied to soil for plant growth and development.

#### **Organic carbon and carbon to nitrogen (C:N) ratio**

The decrease in organic carbon during the vermicomposting process indicates complete degradation, maturity, mineralization and waste decomposition (Hait and Tare 2011). Earthworms and microbes in the feed mixtures activates microbial respiration and degradation of organic wastes, thereby increases the loss of organic carbon during the vermicomposting process (Garg and Kaushik 2005; Suthar 2006). C:N ratio is an important parameter used for determining the vermicompost maturity and stability. The C:N ratio reduction in the final vermicompost indicates rapid mineralization and decomposition of the initial raw material and is mostly used parameter for stability and maturity of organic wastes (Suthar 2008; Garg and Gupta 2011; Singh et al. 2014). According to Suthar and Singh (2008), the loss of carbon and addition of nitrogen during the vermicomposting process reduces the C:N ratio in the end product. A C:N ratio of  $<20$  confirms organic waste mineralization which indicates compost maturity; however, a C:N ratio of  $<12$  is also preferred for agricultural purpose (Shak et al. 2014).

#### **Nitrogen, phosphorus, potassium, sodium and calcium**

In vermicomposting process, the earthworms and microorganisms modifies the chemical properties of initial wastes. The nutrient content in the final vermicompost is generally found to be higher than that in the initial feed stocks. According to Suthar (2007), decaying tissues of dead earthworms enriches the nitrogen content of vermicompost and nitrogen transformation further increases the nitrogen in the vermicomposting process. Yadav and Garg (2011) demonstrate that the addition of nitrogen in final vermicompost is due to the mineralization of carbon-rich materials and decreases in pH and organic

carbon content. According to Pramanik et al. (2007), maximum phosphorus in the final feedstocks of vermicompost is mainly due to acid formation during organic waste decomposition and is responsible for solubilization of insoluble phosphorus. The net loss of dry mass which concentrates the phosphorus in the final feedstocks of vermicomposting also increases the phosphorus content (Ravindran and Sekaran 2010). Many researchers have reported that vermicomposting of organic materials increases the potassium, sodium and calcium concentrations in the final feed mixtures (Sangwan et al. 2010; Bhat et al. 2015a; Lim and Wu 2016). The changes in the nutrient contents (pH, electrical conductivity, organic carbon, C:N ratio, nitrogen, phosphorus, potassium, sodium) between the initial and final vermicompost suggests mineralization of organic wastes by earthworms (Table 2).

#### **Biological evaluation of vermicompost maturity**

##### **Seed germination index**

Germination index which combines the measure of seed germination and root growth of seeds is one of the most sensitive biological parameter used to evaluate toxicity and the degree of maturity and stability of vermicompost (Zuconi et al. 1981; Wong et al., 2001; Campitelli and Ceppi 2008). GI value of more than 70% indicates the good compost/vermicompost maturity (Raj and Antil 2011) whereas a GI below 50% indicates an immature compost/vermicompost (Zuconi et al. 1981). Various researchers have attempted to determine the vermicompost maturity, and the results have revealed that the vermicompost enhanced seed germination (Edwards and Burrows 1988; Bachman and Metzger 2008). Majlessi et al. (2012) used GI as indicator of the stability and maturity of vermicompost produced from food waste. The GI value was 12.8% in the initial mixture food waste and reached a value of 58.4% at the end of the vermicomposting. The increase in GI can be due to the progress of decomposition of organic substrates and reduction of phytotoxic elements resulting from vermicomposting process. Kumar et al. (2013) studied the effect of

**Table 2** Physico-chemical analysis of initial and final cattle dung vermicompost

Parameters	Initial cattle dung	Final vermicompost
pH	$8.35 \pm 0.08$	$7.12 \pm 0.05$
EC (mS/cm)	$3.52 \pm 0.05$	$2.82 \pm 0.03$
Total organic carbon (%)	$46.28 \pm 0.52$	$33.79 \pm 0.36$
C:N ratio	$34.53 \pm 0.26$	$16.89 \pm 0.43$
Total Kjeldhal nitrogen (%)	$1.34 \pm 0.01$	$2.0 \pm 0.03$
Total phosphorus (%)	$0.59 \pm 0.06$	$1.08 \pm 0.05$
Total potassium (%)	$2.23 \pm 0.04$	$1.96 \pm 0.06$
Total sodium (%)	$8.09 \pm 0.28$	$13.42 \pm 0.21$

Source Bhat et al. (2013, 2016b)

vermicompost maturity on the GI of *Vigna mungo* seeds. The results reveal that the vermicompost samples yielded high germination rate (80%) as compared to the control (50%). GI has been widely used for the evaluation of maturity and stability of compost/vermicompost extracts (Warman 1999; Paradelo et al. 2010), because it measures the combined effect of the physical, chemical and biochemical properties. Villar et al. (2016) observed higher GI (97.9%) of vermicompost than that of control. Earthworms were effective for reducing phytotoxic substances, suggesting a suitable level of maturation of final vermicompost produced from municipal sewage sludge. The results suggested that the GI showed that earthworms improved the sludge properties, reaching optimal values of maturation and stabilization. Unuofin et al. (2016) observed over 80% GI for all test crops (tomato, carrot and radish). The results indicated that addition of phosphorus as rock phosphate to cow dung-waste paper mixtures in the presence of earthworms resulted in matured vermicompost and free of phytotoxins.

#### **Relevance and use of sophisticated techniques (SEM, FT-IR, TG and UV-vis spectroscopy) in evaluation of vermicompost maturity**

##### ***Scanning electron microscopy analysis in vermicomposting***

Degradation of the organic substrates by earthworms is reflected by the SEM images. Earthworms ingest and grind the substrate in the gizzard. Further disaggregation by enzymes and microflora act on the substrate occurs during the passage in the gut of the earthworm (Edwards and Bohlen 1996). Various researchers have used SEM technique to observe the changes in surface morphology of pre- and post-vermicomposted organic wastes (Ravindran et al. 2008; Ravindran and Sekaran 2010; Zhao et al. 2010; Li et al. 2011; Unuofin and Mnkeni 2014; Lim et al. 2014, 2015; Kumar et al. 2014; Rajpal et al. 2014; Bhat et al. 2014, 2015a, b; Lim and Wu 2015, 2016; Hussain et al. 2016a).

Ravindran et al. (2008) recorded SEM to know the changes in surface morphology (texture) in the control and final vermicomposted mixtures of a solid waste (animal fleshing) produced from leather industry. The study observed that the aggregates of biomass was firmly bounded in the control animal fleshing mixture, whereas the lignin and the protein matrix was broken down by worms in the final feed mixtures of animal fleshing waste. The numerous surface changes in the post-vermicomposted mixtures of animal fleshing confirmed the compost maturity and degradation of waste by earthworms. Zhao et al. (2010) recorded SEM during the vermicomposting of domestic wastewater sludge. The study reported that the initial sludge had a fluffy structure with maximum rod-shaped cells, whereas the fresh casts produced in the vermifilter exhibited a porous and fragmented appearance. Using

SEM analysis of vermicomposted sewage sludge and cow dung, humic acid-like fraction has been observed by Li et al. (2011). In the initial raw samples, humic acid like-fraction was characterized by flakes and fragments. However, in the final vermiproducts, humic acid-like fraction were close-grained, lumpy and possesses water permeability and ventilation for soil application.

Unuofin and Mnkeni (2014) confirmed the extent of humification using SEM analysis in the vermicomposts of cow dung-waste paper mixtures. The well-humified products and fine grain textures were more produced by the worm stocking densities of 12.5 g worms per kilogram. Lim et al. (2014) studied SEM to identify texture changes in palm oil mill effluent amended with soil or rice straw in the presence of *Eudrilus eugeniae*. The SEM results revealed that the initial raw mixture was characterized by long fibers. However, the final feed mixtures were more porous and fragmented. The study indicated that the earthworms digested and fragmented rice straw leading to more surface area in the final vermicompost. Kumar et al. (2014) evaluated the vermicompost maturity using SEM technique during vermicomposting of flower waste. The study observed that the vermicompost particle size was smaller than the control. The final vermicompost showed numerous surface features than the control which confirms the compost maturity. Rajpal et al. (2014) studied SEM during the vermicomposting of organic fraction of municipal solid waste and sewage. The results revealed that final feed mixtures showed a different physical appearance than the initial raw waste. The initial raw waste was characterized by loosely packed fluffy and rod-shaped cells. However, spherical cell-like structure and reduction in rod-shaped cells were observed in the final feed mixtures. The final vermicompost was completely digested indicating that the complete degradation was observed in the presence of earthworms and microbes.

Bhat et al. (2014) studied SEM to identify surface changes in pressmud sludge mixed with cow dung in the presence of earthworm *Eisenia fetida*. The final vermicomposted samples confirmed the greater number of surface changes which indicates the mineralization of organic wastes. Bhat et al. (2015b) also applied SEM technique to identify the surface changes in the pre- and post-vermicomposted samples of sugar beet mud and pulp in the presence of *E. fetida*. The aggregates of biomass were observed in the initial feed mixtures, whereas in the final feed mixtures, the lignin and protein matrix was defragmented by earthworm *E. fetida* resulting in a high quality vermicompost with high porosity. Lim et al. (2015) studied SEM to characterize changes in initial and final vermicomposted empty fruit bunches. The vermicomposted empty fruit bunches showed a different physical appearance as compared to the pre-waste of empty fruit bunches. The shape of final vermicompost

was more granular with greater surface area. Lim and Wu (2015) also studies SEM to determine the compost maturity derived from palm oil mill effluent. The final vermicompost was found to be more fragmented and scattered in nature than the initial raw wastes and control. Vermicompost maturity of decanter cake derived from palm oil mill using SEM analysis has been observed by Lim and Wu (2016). The final vermicomposted mixtures revealed a more fragmented texture than the initial waste mixtures. Hussain et al. (2016a) studied SEM to identify the disaggregation of salvinia weed during its vermicomposting. The micrographs of the final vermicompost revealed strong disaggregated material as compared to that of the initial mixtures which showed contiguous structures. The SEM images clearly reflect the mineralization of salvinia weed.

#### **Fourier transform infrared spectroscopy analysis in vermicomposting**

Mineralization of organic matter and degradation of complex aromatics (lignin, polyphenols) into simpler compounds (carbohydrates, lipids) by earthworms can be analyzed by FT-IR spectroscopy. FT-IR spectroscopy technique indicates compost maturity/stability and is a promising technique for identification of functional groups in composting/vermicomposting. Sen and Chandra (2007) applied FT-IR spectra to estimate humic acid derived from sugar industry wastes, which showed broad band at 3400–3300  $\text{cm}^{-1}$ . The carboxylic groups and aromatic structures decreased in the final vermicomposted mixtures indicating extensive decomposition during vermicomposting process. Ravindran et al. (2008) applied FT-IR spectroscopy technique to identify the chemical structural changes in the control and final vermicomposted mixtures of a solid waste (animal fleshing) produced from leather industry. The deformation of bands at 1100 and 2925  $\text{cm}^{-1}$  in the final vermicomposted mixtures confirms degradation for cellulose, hemicelluloses, lipid and fat. The FT-IR spectral results confirmed the reduction of polypeptides, polysaccharides and aromatic structures in the final vermicomposted samples indicating extensive mineralization and vermicompost maturity.

FT-IR spectroscopy analysis of non-recyclable paper waste and cow dung has been observed by Gupta and Garg (2009). The FT-IR spectra confirmed reduction in aliphatic compounds in the final vermicomposted mixtures. The band height reduction at 3100–3600  $\text{cm}^{-1}$  was observed in the vermicomposted samples as compared to that in the raw waste. Decrease in peak intensities of final vermicomposted samples at 2783–2879  $\text{cm}^{-1}$  in the aliphatic region confirms decomposition of aliphatic compounds. Deka et al. (2011) applied FT-IR spectroscopy in distillation waste biomass of java citronella (*Cymbopogon winterianus* Jowitt.). The authors confirmed that the FT-

IR spectra of final vermicomposted mixtures revealed increased nitrogen compounds and decreased aliphatic and aromatic compounds as compared to the pre-vermicomposted waste mixtures which indicate degradation of biowaste during vermicomposting process. In vermicomposted samples, the presence of less intensity of peak for  $\text{CH}_2$  and  $\text{CH}_3$  at 2915 and 2846  $\text{cm}^{-1}$  indicates the decrease of aliphatic compounds which indicates the stabilization or decomposition of biowaste material. Ali et al. (2012) applied FT-IR spectroscopy in vegetable waste, tree leaves, saw dust and cow dung mixtures using rotary drum composting process. The results confirmed that the seasonal variation and composting time determines the decrease in aliphatic and polysaccharide components. Lv et al. (2013) applied FT-IR spectra on water extractable organic matter (WEOM) extracted from vermicomposting process of cattle dung. The results indicated a decreasing trend of aliphatic C–H stretching at 2936–2958  $\text{cm}^{-1}$  that confirms lipids and carbohydrate degradation. In general, decrease of aliphatic compounds, N-containing groups, carbohydrates and enrichment of aromatic components in the WEOM confirmed the organics transformation and maturity during the vermicomposting of cattle dung.

FT-IR spectroscopy analysis of *Parthenium* mediated vermicompost has been analyzed by Rajiv et al. (2013). The FT-IR spectra showed functional groups of metabolites which indicates stabilization and degradation of *Parthenium* weed by vermicomposting process. The FT-IR spectra of final vermicomposted mixtures revealed the absence of parthenin toxin and phenols as compared to the initial feed mixture. Ravindran et al. (2013) applied FT-IR spectroscopy for biodegradation of fermented animal fleshing mixed with leaf litter and cow dung using earthworm *E. eugeniae*. The results revealed that the appearance of COO groups and relative reduction in OH,  $\text{CH}_3$  and  $\text{CH}_2$  groups in vermicomposted mixtures indicated decrease in aliphatic compounds and organic waste mineralization. El Ouaquodi et al. (2015) used FT-IR spectroscopy analysis for the evaluation of lignocellulose compost maturity (date palm waste). The infrared spectroscopy revealed an increase in aromaticity and polysaccharide reduction which indicates increase in humification/mineralization during composting process. Hussain et al. (2015) applied FT-IR spectroscopy to identify the toxic constituents of lantana eliminated by vermicomposting. The FT-IR spectra confirmed that the phenols and the sesquiterpene lactones of lantana were destroyed by vermicomposting and the lignin content of lantana was also reduced in the final vermicompost.

Lim and Wu (2016) applied FT-IR spectroscopy for vermicompost maturity of decanter cake produced from palm oil mill. The infrared spectroscopy of initial feed mixtures was characterized by a broad peak at 3282  $\text{cm}^{-1}$  (O–H stretch), 2921 and 2852  $\text{cm}^{-1}$  (C–H stretch), 1743  $\text{cm}^{-1}$

(C=O stretch) and intense peak at  $1031\text{ cm}^{-1}$  (C–O stretch) region. After vermicomposting, the infrared spectra of final feed mixtures showed reductions in 3100–3600, 2921 and  $2852\text{ cm}^{-1}$  region and could be due to the mineralization of carbohydrates and aliphatic compounds. The results revealed that the *E. eugeniae* in vermicomposting of decanter cake helped in the stabilization and mineralization process. Das et al. (2016) applied FT-IR technique to confirm stability of toxic jute mill waste amended with cow dung and vegetable waste employing earthworm species, *Metaphire posthuma*. FTIR spectroscopy analysis results observed a higher maturity and stability of vermicomposted jute mill waste as compared to the traditional (without earthworm) product. The presence of aliphatic C–H stretching, stretching vibration of –OH and HOH bonds, and C–C stretching were highly compatible in the vermicomposted samples of jute mill waste. This indicates the waste degradation and mineralization through vermicomposting process. So, FT-IR spectroscopy is an essential instrument to analyze the quality or maturity of end product after vermicomposting in order to use it as perfect manure.

#### **Thermogravimetric (TG) analysis in vermicomposting**

In vermicomposting, TG analysis reveals the decomposition and net mineralization by earthworms. TG analysis is one of the important parameters in evaluation of vermicompost maturity. Ali et al. (2012) used TG analysis to study compost maturity in vegetable waste, tree leaves, saw dust and cow dung mixtures using rotary drum composting process. The results revealed that the weight loss at two different temperatures in feed mixtures of summer and spring season were 5–10% higher than that of winter season. The authors confirmed that the spring and summer season composting gave higher decomposition and maturity of organic wastes than the winter season. Ravindran et al. (2013) used TG analysis to identify the mass loss for biodegradation of fermented animal fleshing waste mixed with leaf litter and cow dung using earthworm *E. eugeniae*. TG analysis of initial feed mixtures showed maximum mass loss where as lower loss of mass was observed in the final feed mixtures of vermicomposting as these mixtures attained stability. This minimum mass loss may be due to the decomposition of organic waste by worm *E. eugeniae*. The TG curves in the temperature range of 200–500 °C of all feed mixtures showed mineralization and thermal degradation of aromatic structures.

Fernandez-Gomez et al. (2015) observed that the earthworm increases the weight losses of humic acids (HAs) obtained from tomato-plant debris (TD) and paper mill sludge. The changes in the HAs' chemical composition during vermicomposting may be due to the joint action of earthworms and microbes. El Ouaquodi et al. (2015) used thermogravimetric analysis for

evaluation of compost maturity of two wastes (date palm waste with couch-grass clippings and date palm waste alone). The authors observed the reduction of mass loss in both the composts which indicates degradation of aromatic structures, aliphatic compounds and carbohydrates. Lim and Wu (2015) studied TG analysis to determine the vermicompost maturity derived from palm oil industry effluent. The authors observed that the TG curves of final vermicompost had lower loss of mass as compared to the pre-vermicomposted waste of palm oil industry effluent which indicates higher maturity of vermicompost. The total mass losses were 82, 67 and 72% for pre-, final vermicompost and control respectively at temperature of 1000 °C. Lim and Wu (2016) also applied TG analysis for vermicompost maturity of decanter cake produced from palm oil mill. The TG peaks observed losses of organic wastes in the temperature of 200–600 °C. The authors indicated degradation of aromatic compounds, aliphatic structures and carbohydrates. The TG curves of initial waste, final vermicompost and control were found at 438, 468 and 455 °C, respectively. The study indicated that the final vermicompost of decanter cake was more stabilized than the initial wastes. Hussain et al. (2016a) studied TG analysis during vermicomposting of salvinia weed. The TG analysis revealed that the maximum mass loss (75.4%) was observed in the initial substrate whereas in the final vermicompost maximum mass loss was only 66.25%. The results indicated net mineralization during the vermicomposting of salvinia weed.

#### **Ultraviolet-visible (UV-vis) spectroscopy analysis in vermicomposting**

The spectroscopy technique is widely used for determining the maturity in vermicompost material. Gieguzynska et al. (1998) and Sellami et al. (2008) suggested three important regions where exact absorbance could be measured in the UV-vis spectra: (1) the absorption at 280 nm indicates the beginning of the transformation of lignin and aliphatic compounds, (2) the absorption at 472 nm indicates the beginning of organic macromolecules depolymerisation and humification, and (3) the absorption at 664 nm is an indication of a highly humified material with a high degree of aromatic compounds and oxygen content in the stabilization phase. Ravindran et al. (2013) performed UV-vis spectroscopy analysis to assess the degree of humification during vermicomposting of fermented animal fleshing waste. The absorbance ratios ( $Q_{2/6}$ ,  $Q_{4/6}$  and  $Q_{2/4}$ ) in all treatments decreased and reached minimum at the end. The humification index decreased in treated mixtures with earthworms compared to that in the treated mixtures without earthworm. Low humification index in the final vermicompost indicates a high level of organic material humification. Lim and Wu (2015) studied

UV-vis spectroscopy analysis to determine the degree of humification during vermicomposting of palm oil industry effluent. The UV-vis spectra of the final vermicompost showed wider shoulder as compared to initial waste mixtures, reflecting the maturity of the final vermicompost. The results indicated that both the humification index ratios ( $Q_{2/6}$ ,  $Q_{2/4}$ ) were lower in the final vermicompost as compared to initial wastes. The decreases in humification index ratios ( $Q_{2/6}$ ,  $Q_{2/4}$ ) indicates decomposition and degradation of organic compounds by earthworms. Hussain et al. (2016b) studied UV-vis spectroscopy analysis to assess the degree of humification during vermicomposting of toxic parthenium weed. The results reveal that the humification index ( $Q_{4/6}$ ) values of initial parthenium weed (7.29) were reduced (1.89) after vermicomposting. The reduction in the humification index of final vermicomposted parthenium weed indicates weed's transformation into a strongly humified and matured material. Hussain et al. (2016c) also confirmed the high level of humification during the vermicomposting of toxic and allelopathic ipomoea weed. The findings indicated that the humification index ( $Q_{4/6}$ ) values of toxic weed reduced from 7.74 to 1.90 in the final vermicompost. The fall of humification index achieved after vermicomposting of ipomoea weed indicates high level of humification by earthworms. The earthworm along with microorganisms accelerates the decomposition and stabilization of organic material and turns it into stable humus.

## Conclusions

The determination of maturity and stability of vermicompost is essential before it can be used for agricultural application. Use of instrumental techniques such as SEM, FT-IR, TG and UV-vis spectroscopy analysis revealed major beneficial transformations and featured changes in the characteristics of organic wastes during the process of vermicomposting. The physico-chemical and biological parameters also confirmed rapid decomposition and mineralization by earthworms. None of the techniques give us the exact information on the maturity of vermicompost that is why different techniques have been used for evaluation of vermicompost maturity. The results of many authors revealed that these techniques confirmed complete organic matter mineralization which indicates the vermicompost attains maturity and stability and thus can be applied for agricultural purposes. Quality of the end product of any vermicomposting process has been always an important issue for commercial application of this technology. Use of sophisticated techniques/instruments will help to solve the use of end product as good manure for end users.

## Abbreviations

Ca: Calcium; EC: Electrical conductivity; FT-IR: Fourier transform infrared spectroscopy; GI: Germination index; K: Potassium; MPa: Megapascal;  $mScm^{-1}$ : Milli semen per centimetre; N: Nitrogen; Na: Sodium; OC: Organic carbon;

P: Phosphorus; SEM: Scanning electron microscopy; TG: Thermogravimetry; UV-vis: Ultraviolet-visible spectroscopy; WEOM: Water extractable organic matter

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## Authors' contributions

SAB collected and reviewed the literature and drafted the manuscript. JS provided guidance and improved the quality of the manuscript. APV provided guidance and resources, helped in the designing and finalized the manuscript. All authors read and approved final manuscript.

## Competing interests

The authors declare that they have no competing interests.

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