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Research article

Thermophilic ligno-cellulolytic fungi: The future of efficient and rapid bio-waste management

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ABSTRACT

To accelerate the process of decomposition using consortia of thermophilic ligno-cellulolytic fungi, different crop residues *viz.* sorghum (SG), soybean (SS), maize (MS), sugarcane (SC), cotton (CS) and pigeon pea (PS) with a varied C:N ratio and sawdust (SD) having high lignin content were collected and used for decomposition process. Compost quality assessed by evaluating different maturity and stability indices at five succeeding stages [first mesophilic (M1), thermophilic (T), second mesophilic (M2), cooling (C) and humification (H)]. A significant reduction was observed in the C:N ratio, biodegradability index, nitrification index, ratio of water-soluble carbon to organic nitrogen (WSC/Org.N) with an increase in concomitant over time while Ash (%), organic matter loss (%), CEC/TOC ratio, cellulose biodegradation ratio (BR) and lignin/cellulose ratio were significantly increased with time. By correlation study, biodegradability index (BI) and fluorescein diacetate (FDA) hydrolysis emerged as the most suitable compost maturity and stability parameters, respectively. Principal component analysis (PCA) results confirmed that BI, BR, WSC/Org. N and FDA can be regarded as key indicators for assessing compost quality. Our findings conclude that fungal consortia of *Tricoderma viride*, *Rhizomucor pusillus*, *Aspergillus awamori* and *Aspergillus flavus* can accelerate decomposition time from 8 to 12 months (which is normal farming practice) to 120 days.

1. Introduction

World's most important zones devoted to intensive agriculture are located in sub-tropical climates that produce several million tonnes (Mt) of crop residues annually. These residues either burnt or removed from agricultural fields due to scarcity of time, space and manpower. The result is a substantial decline in productivity, soil organic carbon, soil health, and indirect loss of biodiversity, human health problems and climate change. It is estimated that burning of 98.4 Mt of crop residue emits 8.57 Mt CO, 141.15 Mt CO_2, 0.037 Mt SO_x, 0.23 Mt NO_x, 0.12 Mt NH_3 (Jain et al., 2014). In the past few decades, strategies have been adopted for managing the huge amounts of crop residues and these methods include residue incorporation, conservation agriculture, off-farm composting, etc. However, the pace of residue generation is making it challenging to manage it scientifically and effectively. Thus, more emphasis has been given to residue recycling back into the soil because it can stabilize organic matter, reduce environmental pollution and serve as a potential source of soil humus and humic substances (Manna et al., 2018). Surface retention and composting are promising on-farm management options to address the issue of burning as well as maintaining soil health and long-term sustainability of crop productivity.

In general, farmer's practice takes 8–12 months for making compost. Thus, there is an urgent need to produce compost in a shortest possible time that may minimize burning of residues. A few studies

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have attempted to assess compost quality in terms of stability and maturity. Compost maturity refers to its efficiency for plant growth, whereas stability points to the degree or rate of organic matter decomposition through microbial respiration and enzymatic activities (Alvarenga et al., 2016; Bernal et al., 2009a). Microorganisms are known to release different hydrolytic exoenzymes for depolymerization of proteins, lignin, cellulose, lipids and other complex molecules of organic matter (Karadag et al., 2013; Manna et al., 1997). However, the residue's lignin and cellulose content actually determines the rate of decomposition. Some researchers have employed pretreatments such as acid or alkaline solution to remove lignin and hemicellulose from organic crop residues but these are not cost effective for large-scale production of compost. Therefore, the utilization of microbes for accelerating residue decomposition during composting is the most eco-friendly, economical and viable option for recycling crop residue in agriculture.

Microbes are also reported to have the ability to adapt under extreme conditions such as high temperature and low pH. Many microorganisms dominate at various stages of residue decomposition during composting. At moderate temperature, mesophilic microorganisms start the process of decomposition by exothermic breakdown of soluble and readily degradable compounds. The decomposition rate is relatively rapid at 40 °C according to various studies (Bernal et al., 2009b; Huang et al., 2006). Once the temperature exceeds 40 °C, the mesophiles are deactivated and subsequently replaced by thermophilic microbes. The optimum temperature for thermophiles ranges between 50 and 60 °C (Bernal et al., 2009b). Maximum breakdown of recalcitrant materials such as lignin, protein, fat and complex carbohydrates (cellulose, hemicelluloses) occurs during the thermophilic stage. Once all the complex molecules are broken down, the temperature reduces and mesophilic microorganisms again predominate during the maturation phase (curing phase) for the remaining organic matter decomposition and stabilization (Manna et al., 2017a, 2017b). The significance of this thermophilic stage of composting provide us an opportunity to manipulate it to accelerate the decomposition rate and substantially reduce the composting time.

Therefore, extensive research is required to increase the duration of the thermophilic stage for faster breakdown of complex molecules by externally introducing the thermophilic lignocellulolytic microbial consortia. The present study has the following objectives: (i) set up a staggered bioaugmentation process using a thermophillic lignocellulolytic microbial consortia for the acceleration of residue decomposition; (ii) assess compost quality through maturity and stability indicators of humification and stabilization; and (iii) derive new maturity and stability indicators linked to accelerated decomposition.

2. Materials and methods

2.1. Fungal consortia preparation

Approximately one hundred isolates were collected from Bhanpur dumping site ($23^0 17' 51.74'' N$, $77^0 26' 9.10'' E$), Bhopal, India. In which, four fungal isolates were screened positive for thermophilic and ligno-cellulolytic potential. Lignolytic activity was determined by utilizing the plate assay method (Sundman and Näse, 1972) and cellulase production was analysed on modified carboxymethyl cellulose (CMC) plates flooded with Grams Iodine (2g KI, 1g Iodine, 300 mL distilled water) (Sahu et al., 2018). All microbes were checked for their thermophilic potential by incubating at 60 °C. Screened fungal isolates were identified by Institute of Microbial Technology (IMTECH), Chandigarh, India, as *Tricoderma viride*, *Rhizomucor pusillus*, *Aspergillus awamori*and *Aspergillus flavus*. 0.5 g of each fungal inoculum containing 10^5 viable cell/mL was used.

2.2. Composting materials

An experiment was conducted to accelerate the decomposition of crop residue in the laboratory of the Soil Biology Division, ICAR-IISS, Bhopal, India. The crop residues (2–5 cm) of sorghum (*Sorghum bicolor*) (SG), soybean (*Glycine max*) (SS), maize (*Zea mays*) (MS), sugarcane (*Saccharum officinarum*) (SC), cotton (*Gossypium* sp.) (CS) and pigeon pea (*Cajanus cajan*) (PS) used in this research were collected from local agricultural fields which are regularly cultivated by farmers of central India. In this study, sawdust (SD) obtained from the wood industry in central India, was used because it has higher lignin content and wide C:N ratio as compared to low lignin materials.

The initial chemical properties of the crop residues were analysed (Table S1). Two sets of experiments were set up. One set up consisted of about 2 kg of sterilized crop residues inoculated with 0.5 kg of fresh cowdung slurry containing the consortia of microbes. The moisture content was gravimetrically maintained at 60% with distilled water. The second set up for this experiment was the non-inoculated treatment i.e. without fungal consortia.

2.3. Treatment design

Both sets of experiments were done in triplicate. Plastic pots were embedded in an aerobic controlled state for the composting process that could maintain temperature at about 30–40 °C for 7 days. The aeration rate was controlled by an intelligent control system (aeration rate set to 0.80 L $min^{-1}kg^{-1}$material). There were two holes closed with sterilized cotton at the top of the pot for aeration and uniform distribution of gas. The duration of the experiment was 120 days.

2.4. Sampling and analysis

Sampling was done from three positions (top, middle and bottom) of the container at 7, 30, 60, 90 and 120 days of incubation, denoted as first mesophilic (M1), thermophilic (T), second mesophilic (M2), cooling (C) and humification (H) stages, respectively. The samples were uniformly mixed and homogenized. The representative sample was subdivided into three parts. In which, first part was used to determine the moisture content by calculating the loss of a sample weight at 105 °C for 24 h in hot air oven.

The second part of the sample was air-dried and processed (passed through a 2 mm mesh) to analyze pH, EC, and total nitrogen (TN). Organic matter content and total organic carbon (TOC) were calculated by recording the weight loss in a muffle furnace at 600 °C for 8 h (Waggoner, 1974). Cellulose and lignin were determined using the acid detergent fibre method (Rowland and Roberts, 1994). Cation exchange capacity (CEC) was assessed by modifying the $BaCl_2$-triethanolamine method (Lax et al., 1986). Water soluble carbohydrates (WSCarb.) were determined by the anthrone method (Brink et al., 1960) and the water soluble carbon was assessed via centrifugation (McGill et al., 1986). Total nitrogen (TN), ammonium nitrogen (NH_4-N) and nitrate nitrogen (NO_3-N) were determined by the digestion and distillation method (Jackson, 2005). The organic nitrogen (Org.N) was computed by establishing the difference between TN and the inorganic nitrogen (sum of NH_4-N and NO_3-N), and the nitrification index was derived as the ratio of NH_4-N and NO_3-N.

The third part was stored at 4 °C for biological analysis. The microbial abundance at different stages of decomposition was calculated by counting the CFU (colony forming units) on respective media (Vargas-Garcia et al., 2010). Enzyme activities such as dehydrogenase (DHA) (Casida et al., 1964), β-glucosidase (Eivazi and Tabatabai, 1988) and fluorescein diacetate (FDA) hydrolysis were assessed (Adam and Duncan, 2001).

The organic matter's mineralization rate was assessed by measuring organic matter loss at different stages of composting, according to equation 1 (Antil et al., 2012)

$$\text{Organic matter loss}(\%) = \left[(X1 - X2)/X1\right] \text{x}100 \quad [1]$$

where *X1* is the percentage of organic matter at initial stage and *X2* is the percentage of organic matter at nth day of sampling.

Biodegradation ratio (BR) of cellulose fractions was computed according to equation 2 (Wang et al., 2011)

$$BR = \left(\frac{m_0 - m_n}{m_0}\right) \times 100 \quad [2]$$

where m_0 is the initial cellulose content and m_n is the cellulose content at nth sampling day.

The biodegradability index was determined by using equation 3 (Morel et al., 1979)

$$\text{BI} = 3.166 + 0.039\ \text{TOC} + 0.832\ \text{WSCarb.} - 0.011\ \text{days} \quad [3]$$

where BI is the biodegradability index, TOC is total organic carbon and WSCarb. is the water soluble carbohydrate.

During decomposition process, organic matter degradation was expressed as the first-order kinetic model (Eq. (4)) (Bustamante et al., 2008)

$$\text{OM loss}(\%) = \text{A}(1 - e^{-kt}) \quad [4]$$

where *A* represents maximum organic matter degradation (%), *k* refers to the rate constant and *t* is the composting time.

2.5. Data analysis and statistics

The pot experiments were studied in triplicate. The values reported in the tables are mean ± SD (standard deviation). Furthermore, the data were subjected to two-way analysis of variance (ANOVA). The fit of the first order kinetic model to the experiment results was indicated by calculating the R^2 values. Pearson correlation coefficients were calculated between the stability and maturity parameters. Principal component analysis (PCA) was conducted to identify the best indicator of compost quality. Duncans multiple range test was performed to further elucidate differences among means ($p < 0.05$). All statistical analyses were done using SPSS 16.0 software. Figures were represented in circular graph by Circos (Krzywinski et al., 2009).

3. Results and discussion

3.1. Changes in physical characteristics

Compost samples have been evaluated for physical, chemical and biological properties. Referring to the physical properties, the raw materials' textures gradually changed during the initial days of the composting process, followed by the formation of humic substances at the humification stage (H). The mature compost had a bulk density of 0.9–1.8g cc^{-1}. At the maturity stage, moisture content varied from 25 to 30% (w/w). It appeared to be an important factor for microbial activity, as less moisture content inhibits the growth of beneficial microorganisms, whereas excess moisture leads to toxic volatile substances being produced and unpleasant odors as a consequence of anaerobic conditions. The weight loss was significantly more during the first mesophilic stage (M1), which agrees with other studies' results (Gautam et al., 2010; Pan et al., 2012).

3.2. Changes in chemical characteristics

3.2.1. Maturity indices

3.2.1.1. Total organic carbon (TOC) and total nitrogen (TN) ratio The effects of inoculation on TOC and C:N ratio at different stages of composting are presented in Table S2. The TOC content of different crop residues ranged between 45.2 and 50.4%. As decomposition time progressed, a significant decrease of TOC was recorded irrespective of crop residues and inoculation. The lowest TOC was recorded in the SG. Among the different stages a sharp decrement in TOC was observed during M1 and T in the inoculated samples (Table 1). This decline occurred due to the degradation of organic matter (OM) as the microbes decomposed 60–70% of carbohydrates and the carbon was lost as gas in the form of CO_2 (Zhou et al., 2016). The percentage of TOC of used crop residues fell drastically (Fig. 1). The percent reduction was noted highest in PS (19.4%) while the lowest occurred in SD (8.9%) with inoculation of microbial consortia. With progressive reduction in TOC as well as bulkiness of the crop residues, the TN revealed an upward trend in successive composting stages. Further, the more substantial increase in TN was recorded in treatments inoculated with ligno-cellulolytic fungi in almost all the crop residues.

The C:N ratio has been considered one of the most important factors of compost quality and a good indicator of OM decomposition. The initial values of C:N ratios of the crop residues (Table S1) were 72.8:1 (SG), 52.11:1 (SS), 81.17:1 (SD), 78.14:1 (MS), 74.85:1 (SC), 79.37:1 (CS), and 55.54:1 (PS). In this study, a sharp fall in the mean C:N ratio was recorded in the M1 stage followed by T and other subsequent stages and remained stable after H stage (Table 1, Fig. 1). Moreover, the treatments with inoculation of microbial consortia, the faster decomposition rate and reduction in C:N ratio were noted in the 15:1–22:1 range at H stage, depending on the substrate. The significant interaction between inoculation, crop residues and stages was recorded which indicated that applied microbial consortia enhanced the composting process (Table S2). Out of the seven crop residues, lower mean C:N ratio was recorded in SS (21.82) and PS (22.93), thus confirming a higher decomposition rate when inoculated (Table 1).

The rapid transformation of organic carbon and concomitant release of carbon dioxide gas, followed by an increase in nitrogen content per unit material and decrease in the organic acid content is one possible explanation for the frequent drop in C:N ratio. Our results corroborated with those documented in other studies, where it was reported that the C:N ratio of the mature compost varied from: < 12:1 to < 25:1 [< 12 (Bernal et al., 1998b); < 15 (Bernal et al., 2009a; Morel et al., 1979); 15–20 (Swarnam et al., 2016); < 20 (FCO, 2013; Golueke, 1981; Manna et al., 2012; Zhou et al., 2016); ≤25 (CCME, 2005; TMECC, 2002)].

3.2.1.2. Organic nitrogen mineralization and nitrification index With decomposition, ammonium nitrogen (NH_4-N) decreased gradually but a substantial increase in nitrate nitrogen (NO_3-N) was recorded in all residues. At humification (H) stage, NH_4-N values were recorded in the range of 0.6–0.85 g kg^{-1} (non-inoculated) and between 0.20 and 0.39 g kg^{-1} in inoculated treatments. Thus, the maximum limit (< 0.4 g kg^{-1}) of NH_4-N suggested for mature compost has been reached at H stage (Zucconi and Bertoldi, 1987). The apparent decline in NH_4-N might be due to ammonia volatilization and nitrification at high temperature and high pH (Raj and Antil, 2011; Wang et al., 2013). Conversely, a drastic increase in the NO_3-N concentrations was recorded during decomposition up to M stage. At H stage, NO_3-N content ranged between 2.28 and 2.56 g kg^{-1}. Ammonification that took place during composting process, released NH_4-N, which further underwent oxidation to increase NO_3-N (Qian et al., 2014). Similar observa-

Table 1
Substrate and stage mean effect of inoculation on maturity indices.

	TOC%	C:N	WSC/Org.N	BI	Nitri.index	Ash%	OM loss%	CEC/TOC	BR Cel.	Lig/Cel.
Crop residues										
SG	25.27 ± 5.26a	30.08 ± 11.8c	1.33 ± 1.13c	3.79 ± 0.88a	0.37 ± 0.15c	54.51 ± 9.43d	45.60 ± 11.3e	2.68 ± 1.14g	23.25 ± 10.7b	0.97 ± 0.24d
SS	28.04 ± 7.25c	21.82 ± 6.88a	0.85 ± 0.42b	3.98 ± 1.01cd	0.30 ± 0.10a	49.51 ± 13.0b	43.13 ± 14.7c	1.80 ± 1.17a	39.43 ± 14.5g	0.96 ± 0.32d
SD	35.15 ± 7.53d	44.26 ± 19.9g	1.78 ± 1.30f	4.29 ± 1.02e	0.40 ± 0.17d	36.73 ± 13.5a	27.83 ± 15.4a	1.99 ± 0.89c	27.88 ± 13.0e	1.08 ± 0.29e
MS	27.91 ± 9.57c	35.26 ± 16.9e	1.71 ± 1.22e	3.91 ± 1.05bc	0.37 ± 0.18c	49.75 ± 17.2b	44.64 ± 18.9d	2.22 ± 1.20e	28.64 ± 15.1f	0.86 ± 0.27c
SC	26.53 ± 7.54b	32.07 ± 16.3d	1.41 ± 1.13d	3.95 ± 1.05bcd	0.37 ± 0.15c	52.24 ± 13.6c	42.28 ± 16.4c	2.32 ± 1.16f	26.11 ± 10.2c	0.76 ± 0.18b
CS	27.73 ± 8.43c	38.48 ± 19.2f	1.84 ± 1.39g	4.02 ± 1.08d	0.29 ± 0.09a	50.09 ± 15.2b	38.71 ± 18.7b	1.90 ± 1.03b	26.67 ± 8.49d	0.87 ± 0.19c
PS	26.48 ± 7.97b	22.93 ± 6.50b	0.74 ± 0.51a	3.87 ± 0.98ab	0.36 ± 0.14b	52.34 ± 12.5c	46.70 ± 13.9f	2.05 ± 1.09d	19.19 ± 7.72a	0.57 ± 0.14a
Stages										
M1	40.53 ± 4.11e	55.89 ± 16.4e	3.20 ± 1.12e	5.47 ± 0.24e	0.52 ± 0.07e	27.03 ± 7.25a	15.47 ± 8.10a	0.78 ± 0.14a	9.62 ± 3.24a	0.53 ± 0.10a
T	31.35 ± 3.54d	36.64 ± 9.92d	1.49 ± 0.42d	4.60 ± 0.22d	0.47 ± 0.07d	43.57 ± 6.30b	34.63 ± 6.85b	1.43 ± 0.33b	21.47 ± 5.94b	0.73 ± 0.12b
M2	25.63 ± 3.62c	27.70 ± 6.40c	1.05 ± 0.31c	3.85 ± 0.19c	0.35 ± 0.04c	53.56 ± 6.63c	46.57 ± 7.10c	1.97 ± 0.42c	29.49 ± 6.19c	0.88 ± 0.15c
C	22.78 ± 3.26b	22.03 ± 3.99b	0.64 ± 0.18b	3.25 ± 0.16b	0.25 ± 0.02b	58.99 ± 6.18d	52.47 ± 7.16d	2.72 ± 0.41d	35.90 ± 8.11d	1.04 ± 0.19d
H	20.49 ± 2.48a	18.37 ± 2.50a	0.50 ± 0.15a	2.71 ± 0.13a	0.15 ± 0.01a	63.10 ± 4.37e	57.24 ± 4.82e	3.79 ± 0.32e	40.08 ± 8.76e	1.17 ± 0.24e

SG: Sorghum straw, SS: Soybean straw, SD: Sawdust, MS: Maize straw, SC: Sugarcane straw, CS: Cotton stalk, PS: Pigeonpea straw, M1: First mesophilic stage, T: Thermophilic stage, M2: Second mesophilic stage, C: Cooling stage, H: Humification stage, TOC: Total Organic Carbon, WSC: Water Soluble Carbon, Org. N: Organic nitrogen, BI: Biodegradability index, Nitri. index: Nitrification index, OM: Organic matter, CEC: Cation Exchange Capacity, BR: Cellulose biodegradation ratio: Lig: Lignin, Cel:Cellulose. Values in the same column are mean ± standard deviation followed by a different lower-case letters are significantly different at $P < 0.05$ according to Duncan's Multiple Range Test.

Table 2
Substrate and stage effect of inoculation on stability indices.

	Bac.	Fun.	Act.	DHA	FDA	Gluco
Crop residues						
SG	4.22 ± 1.23c	2.90 ± 0.90e	2.82 ± 0.52c	2.15 ± 0.69a	88.57 ± 32.36e	139.77 ± 30.01e
SS	4.88 ± 1.40d	2.78 ± 0.65d	3.34 ± 0.66d	1.15 ± 0.53e	114.04 ± 62.11a	130.25 ± 36.88c
SD	3.78 ± 1.12a	2.34 ± 0.69a	2.06 ± 0.31a	1.63 ± 0.70d	101.63 ± 64.51c	132.28 ± 28.10cd
MS	3.90 ± 0.99b	2.50 ± 0.55b	3.52 ± 0.70e	1.58 ± 0.52c	98.17 ± 51.48b	131.85 ± 36.47cd
SC	5.06 ± 1.65e	3.28 ± 0.63f	3.76 ± 0.81f	2.88 ± 0.95f	124.87 ± 68.51g	124.58 ± 30.01b
CS	3.92 ± 0.80b	2.58 ± 0.64c	2.58 ± 0.53b	2.02 ± 0.72b	92.36 ± 49.12d	134.22 ± 33.69d
PS	5.60 ± 1.92f	3.48 ± 0.77g	4.28 ± 1.18g	2.46 ± 0.80b	92.45 ± 45.13f	119.82 ± 33.79a
Stages						
M1	3.75 ± 0.29b	3.88 ± 0.39e	2.73 ± 0.52b	1.01 ± 0.35a	40.13 ± 8.62a	83.93 ± 11.06a
T	6.71 ± 1.12e	2.29 ± 0.31b	4.29 ± 1.18e	1.67 ± 0.56c	93.12 ± 11.82b	133.24 ± 14.95c
M2	4.76 ± 1.09d	3.19 ± 0.57d	3.49 ± 0.81d	2.90 ± 0.66e	194.22 ± 35.91e	179.32 ± 10.08e
C	3.90 ± 0.60c	2.63 ± 0.52c	2.90 ± 0.63c	2.34 ± 0.48d	99.02 ± 16.57d	135.89 ± 7.85d
H	3.27 ± 0.55a	2.20 ± 0.51a	2.57 ± 0.55a	1.99 ± 0.90b	82.13 ± 12.47c	119.61 ± 5.41b

SG: Sorghum straw, SS: Soybean straw, SD: Sawdust, MS: Maize straw, SC: Sugarcane straw, CS: Cotton stalk, PS: Pigeonpea straw, M1: First mesophilic stage, T: Thermophilic stage, M2: Second mesophilic stage, C: Cooling stage, H: Humification stage, Bac.:Bacteria ($\times 10^6$ cfu/g), Fun.:Fungi ($\times 10^4$ cfu/g), Act.:Actinomycetes ($\times 10^5$ cfu/g), DHA: Dehydrogenase activity (mg TPF g^{-1} d^{-1}), FDA: Flourescein diacetate hydrolytic activity (μg flourescein g^{-1} h^{-1}), Gluco: β-Glucosidase (μg PNP g^{-1} h^{-1}).
Values in the same column are mean ± standard deviation followed by a different lower-case letters are significantly different at $P < 0.05$ according to Duncan's Multiple Range Test.

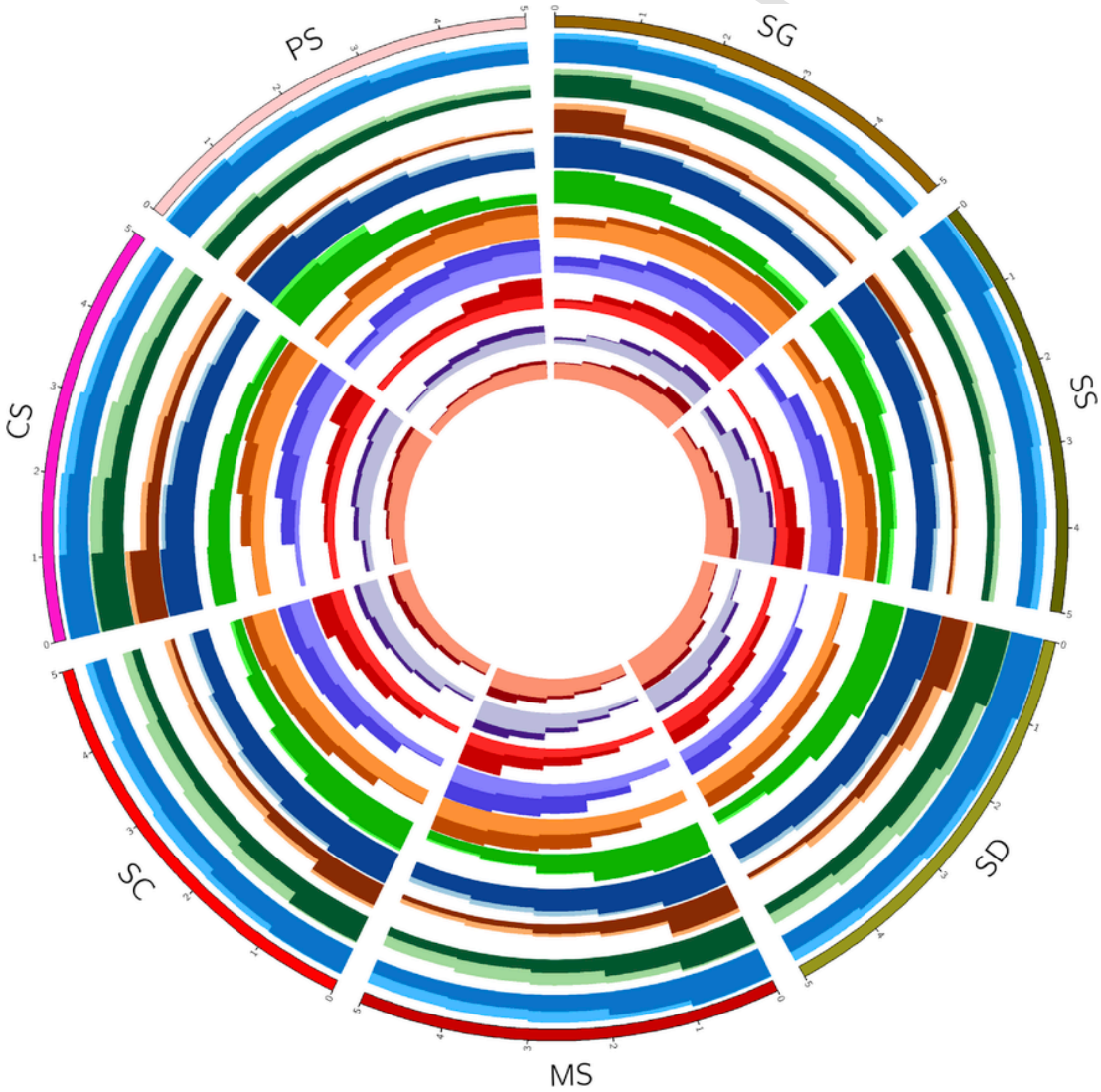

Fig. 1. Maturity indices with decomposition time. The tracks from outside to inside are; Total Organic Carbon (max.-50) pblue; Carbon:Nitrogen ratio (max.-80) green; Water Soluble Carbon:Organic nitrogen (max.-5.5) orange; Biodegradibility index (max.-6) blue; Nitrification index (max.-0.65) pgreen; Ash% (max.-70) porange; Organic matter loss% (max.-65) ppurple; Cation Exchange Capacity: Total organic carbon (max.-4.5) pred; Cellulose biodegradation ratio (max.-56) purple; Lignin:Cellulose (max.-1.5) dorange. Non-inoculated (light colored), Inoculated (dark colored). 0–1:First mesophilic stage (M1); 1–2:Thermophilic stage (T); 2–3:Second mesophilic stage (M2); 3–4:Cooling stage (C); 4–5:Humification stage (H). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

tions have been reported in other studies (Wang et al., 2016; Wu et al., 2010).

Furthermore, the nitrification index (NH_4-N to NO_3-N ratio) has been used as an important index of compost maturity (Bernal et al., 1998b), since during the decomposition process rapid mineralization by ammonifiers and nitrifiers of organic nitrogen takes place. As seen in Fig. 1, the nitrification index ratio decreased gradually, during the first three stages of decomposition due to the larger nitrate concentration at these stages. At H stage of composting in the inoculated treatments, mean nitrification index of all crop residues reached a value of less than 0.16 (Table 1). Thus, it can be inferred that the excessive biological degradation has been reduced and the mature compost is ready to use (Bernal et al., 1998a).

3.2.1.3. Ratio of water soluble carbon (WSC) to organic nitrogen (Org. N) During decomposition, the ratio of WSC to Org. N decreased significantly. More decline has been observed in the inoculated treatment than non-inoculated one. Lowest mean WSC/Org. N was observed in PS (Table 1). At the initial stages, the values of WSC/Org. N ratio were very high (4.43–1.47) which decreased to the 0.76–0.30 range in the inoculated treatment at the H stage and the decline in percentage terms was 76–86% (Table S2). On the other hand, in the non-inoculated treatment the ratio ranged from 1.90-0.65 at H stage. After the decomposition of easily degradable organic molecules, microbes attack and break the complex organic materials from where they derive their energy and nutrients. In the inoculated treatments, at the end of the H stage the mean ratio has been observed as 0.50 (Table 1). According to Bernal et al. (1998a), if WSC/Org. N ratio values is less than 0.55, then the compost is considered to be well matured.

3.2.1.4. Biodegradability index The biodegradability index (BI) measures the level of maturity by calculating hot water soluble carbohydrates (WSCarb.), TOC and composting stages in terms of days using equation (3) (Morel et al., 1979). In this experiment, inoculated crop residues showed BI values between 2.63 and 2.98 at H stage and non-inoculated crop residues ranged from 3.20 to 3.77, thus suggesting immaturity (Table S2). Morel et al. (1979) proposed that the BI value for the mature compost must be < 2.4, yet Garcia et al. (1992) suggested that if BI is greater than 2.9, the compost had not undergone maturation. Hence, it was concluded that inoculated treatment reached maturation, as the mean BI reached 2.71 at H stage (Table 1).

3.2.1.5. Ash% and organic matter (OM) loss% During the decomposition period, ash% significantly increased irrespective of crop residues and stages (Fig. 1). However, the percentage increase of ash was more in inoculated treatments (54–67%) than the non-inoculated treatments (28–46%). Nonetheless in all crop residues the OM loss percentage rose over time due to the availability of easily degradable compounds for microbes (Table 1). A greater loss in percentage terms was observed during the initial stages of composting for all crop residues, and only slowed downin the later stages. Loss of OM was recorded to be significantly higher with fungal inoculation. Furthermore, the OM loss followed a first-order kinetic equation and the parameter values obtained after curve fitting are presented in Table 3. The kinetics of OM loss of inoculated treatments significantly fitted this equation better than the non-inoculated ones, as shown by the R^2 values and rate constant (k) values. These *A* values (52.2–65.9) of inoculated treatments were within the range found by earlier researchers (Bustamante et al., 2008).

3.2.1.6. Cellulose and lignin degradation and lignin:cellulose ratio With reference to cellulose, degradation ratio in the non-inoculated treatment (Table S2) found that most of the cellulose was degraded after the thermophilic stage. In the inoculated treatments, however, biodegradation started in the earlier stages. The percentage of initial and final biodegradation ratio of cellulose in the inoculated and non-inoculated treatments were in the 17.54–33.92% and 11.98–23.00% ranges, respectively. The percentage degradation was highest in the CS (Fig. 1). Hence, it can be stated here that the microbial inoculation accelerated the decomposition process by degrading the recalcitrant fractions.

Table 3
Parameter values of the first-order equation describing organic matter (OM) degradation (SE in parentheses).

		A (%)	*k (days^{-1})*	R^2
SG	**NI**	33.9 (1.02)	0.045 (0.006)	0.992
	I	52.2 (3.47)	0.078 (0.026)	0.938
SS	**NI**	39.6 (5.22)	0.016 (0.004)	0.983
	I	55.3 (2.14)	0.042 (0.006)	0.988
SD	**NI**	45.2 (5.5)	0.005 (0.006)	0.946
	I	58.6 (8.4)	0.013 (0.003)	0.989
MS	**NI**	25.5 (2.08)	0.087 (0.037)	0.905
	I	65.9 (2.76)	0.026 (0.003)	0.994
SC	**NI**	39.5 (19.8)	0.013 (0.012)	0.822
	I	57.5 (1.5)	0.034 (0.003)	0.996
CS	**NI**	46.5 (21.2)	0.009 (0.005)	0.960
	I	59.6 (1.91)	0.024 (0.002)	0.997
PS	**NI**	38.5 (5.0)	0.026 (0.010)	0.937
	I	56.71 (3.1)	0.054 (0.013)	0.968

SG: Sorghum straw, SS: Soybean straw, SD: Sawdust, MS: Maize straw, SC: Sugarcane straw, CS: Cotton stalk, PS: Pigeonpea straw, NI: non-inoculululated, I: Inoculated.
A: Maximum degradation of OM (%), *k:* Rate constant.

A perusal of data in Table 1 shows that the mean values of lignin/cellulose ratio were significantly increased with decomposition and the increment was recorded as being higher in H stage. Similar trend was observed in the earlier studies (Manna et al., 2000). Amongst the different crop residues, the mean ratio was in the range of 0.57–1.08. Specifically, a greater percentage increase was observed in MS (82.34%) and less was recorded in CS (48.15%) when using microbial inoculation. The significant interaction between crop residues, stages and inoculation was recorded (Table 1) which indicated that applied microbial consortia enhanced or accelerated the composting process. It was also observed that higher cellulose content was recorded in cotton compost, which indicated that cotton stalks were less susceptible or more resistant to attack by the microbes. Thus more time is required for its decomposition.

3.2.1.7. CEC/TOC ratio The CEC/TOC ratio is considered to be a good humification index in manures. At H stage CEC was recorded in the range of 72–84 which is greater than the value of 60 meq/100g noted for matured compost in earlier studies (Harada and Inoko, 1980; Karak et al., 2013). Regardless of the crop residue characteristics mean CEC/TOC ratio increased during decomposition stages (Table 1). This might be due to two things:a decrease in TOC caused by rapid mineralization of crop residues and increase in CEC caused by oxidation of molecules and radicals. Moreover, at humification (H) stage, the compost consists of more functional groups per unit carbon indicating more stable organic matter (Antil et al., 2012). However, throughout the composting phase the increment was recorded significantly higher in inoculated treatment (Fig. 1). The CEC/TOC can be considered as a useful maturity index since at the end of humification (H) stage all inoculated crop residues reached similar values (3.27–4.18) (Table S2) irrespective of crop residues (Garcia et al., 1992). The results of the study agreed with those reported by Lax et al. (1986). The effectiveness of microbes used for accelerating the decomposition and humification was confirmed with the CEC/TOC ratio.

The initial and final concentrations for ash%, TOC%, C:N ratio, OM loss%, WSC/Org. N, lig/cel ratios and changes of each parameter (as difference) in inoculated and non-inoculated treatments were calculated (Table S4). For every substrate, a percentage change was higher in the inoculated treatment except for OM loss%.

3.2.2. Stability indices

Biological tests, specifically changes in microbial abundance and enzyme activities were done to evaluate compost stability.

3.2.2.1. Changes in microbial abundance The changes in microbial abundance during decomposition of crop residues are illustrated in Fig. 2. It was found that the bacterial count increased with time notably up to thermophilic stage (T) (Table 2). In non-inoculated treatment the bacterial and actinomycetes biomass showed a similar pattern but biomass was comparatively less than the inoculated one (Table S3). Regarding the crop residues, highest mean population of bacterial (5.6×10^6 cfu/g compost), fungal (3.48×10^4 cfu/g compost) and actinomycetes (4.28×10^5 cfu/g compost) was observed in PS. Bacterial and actinomycetes count was recorded as being the highest at T stage (Table 2).

The fungal biomass, on the other hand, was observed more in first mesophilic (M1) followed by second mesophilic stage (M2) (Fig. 2). A rise in temperature (50 °C) means that a fungal population becomes less active but the biomass will be reactivated when the temperature falls to 35 °C and below (Liu et al., 2011). In our experiment during the initial phase, due to degradation of organic matter, organic acids were released, which lowered pH, thus favoring fungal biomass. Furthermore, it is fascinating to see that even in the fungal inoculum treatment, the bacterial population was more numerous than the fungal population. This may have been due to the dominance of thermophilic bacteria (*Bacillus* sps.) and actinomycetes, especially formed during the thermophilic stage of composting. The diversity of fungi during the decomposition process has also been reported in our earlier study (Manna et al., 2017b). This outcome corroborated with one report that the population of thermophilic bacteria reached its maximumat 14 days of composting and then declined (Goyal et al., 2005). However, there are contradictory findings on the population of microbes during different stages of decomposition (Manna et al., 2017b).

The succession of ligno-cellulolytic fungi as well as bacterial and actinomycetes biomass reached the peak values during the early stages of decomposition process and then declined till H stage. These outcomes might be explained as the presence of ample quantity of water-soluble carbon fractions at the early stage of decomposition which were highly preferred by microbes (Awasthi et al., 2014; Vargas-Garcia et al., 2010).

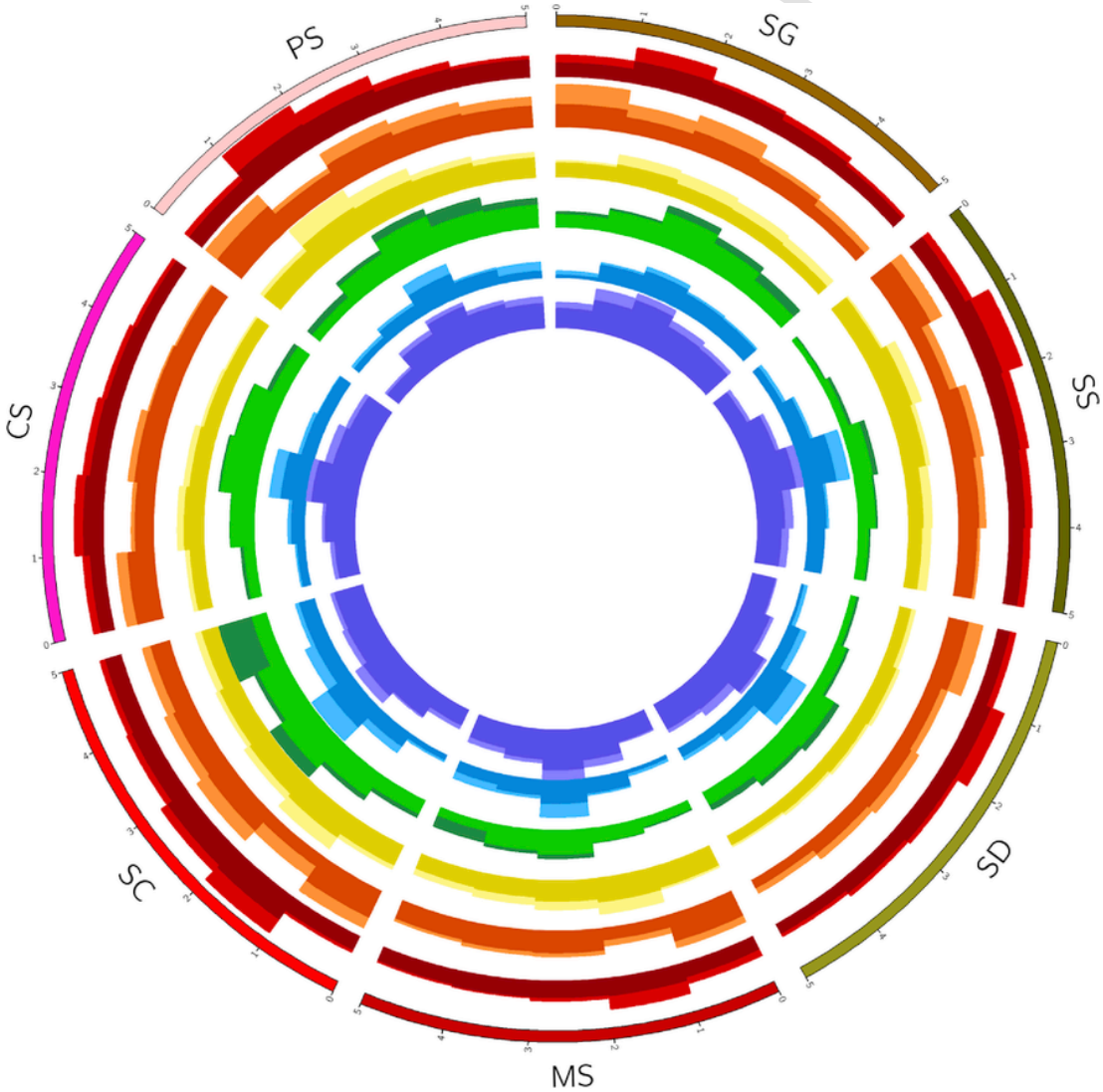

Fig. 2. Stability indices with decomposition time. The tracks from outside to inside are; Bacterial cfu/g X10^6 (max-9); Fungal cfu/g X10^4 (max-5); Actinomycetes cfu/g X10^5 (max - 6.5); DHA (mgTPF g^{-1} d^{-1}) (max-4); FDA (μg fluorescein g^{-1} h^{-1}) (max-250); β-Glucosidase (mg PNP g^{-1} h^{-1}) (max-190). Non-inoculated (light colored), Inoculated (dark colored). 0–1:First mesophilic stage (M1); 1–2:Thermophilic stage (T); 2–3:Second mesophilic stage (M2); 3–4:Cooling stage (C); 4–5:Humification stage (H). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

3.2.2.2. Microbial enzyme activities The decomposition process is solely mediated by microbes, excreting extracellular enzymes. Thus, quantification and characterization of these enzymes provides a better view of carbon, nitrogen transformation and OM decomposition. It is reported by many researchers that microbes involved in the decomposition processes produce more hydrolytic enzymes.

3.3. Dehydrogenase activity (DHA)

Dehydrogenase activity is directly involved in the respiratory chain and thus considered to be a better indicator of microbial activity (Nikaeen et al., 2015; Vargas-Garcia et al., 2010). More dehydrogenase activity (DHA) was recorded (0.95–2.62 μg TPF g^{-1} d^{-1}) in the inoculated treatments in comparison to the non-inoculated one (Fig. 2, Table S3). Amongst 7 crop residues, SC showed highest mean DHA (2.88 mg TPF g^{-1} d^{-1}) at M2 stage of composting (Table 2). During the initial stages of decomposition, microbes excreted more enzymes to catalyze the oxidation of labile organic compounds (Vargas-Garcia et al., 2010). Significant variations in DHA activity have been observed during the composting stages. The decline in activity at H stage illustrated the depletion of possible sources of carbon and energy used by microbes, which ultimately indicates stability and maturity of compost (Nikaeen et al., 2015). The evolution of DHA during composting has therefore been proposed as one of the indicators of organic matter stabilization.

3.4. Hydrolysis of flourescein di acetate (FDA)

The dynamics of substrate degradation can be better indicated by activities of hydrolytic enzymes. Flourescein diacetate (FDA) hydrolysis has been recently used as a sensitive method for evaluating total microbial activity during composting (Nikaeen et al., 2015; Wang et al., 2016). FDA hydrolysis was significantly higher in the inoculated treatment than the non-inoculated one (Fig. 2, Table S3). Amongst crop residues, SC showed the highest FDA hydrolysis (141.66 μg flourescein $g^{-1}h^{-1}$). The FDA hydrolytic activity increased significantly up to M2 stage (194.22 μg flourescein g^{-1} h^{-1}) and then slowly decreased at further stages (Table 2). This can be explained as during the initial stage of decomposition copious amounts of labile organic matter are present and consequently more hydrolysis takes place. Insufficient nutrients caused a gradual decrease in enzyme activity at the end of the decomposition, which indicates stability in composting.

3.5. β-Glucosidase activity

Cellulose degradation is governed by a very important enzyme called β-Glucosidase. During decomposition the activity of the β-Glucosidase enzyme is represented in Fig. 2. The enzyme activity was significantly higher in inoculated treatments than that of the control. This shows that the inoculants promoted the β-glucosidase enzyme activity, and thus degradation of cellulose. The enzyme activity was reached its highest in SG (139.77 μg PNP g^{-1} h^{-1}). The gradual increase in this activity was recorded up to M2 stage (179.32 μg PNP g^{-1} h^{-1}) and then fell till maturation was achieved (Table 2).

3.6. Pearson's correlation coefficients and Principal component analysis

Pearson's correlation coefficients were calculated between maturity and stability indices (Table 4). The TOC, C:N ratio, BI, nitrification index and lignin/cellulose were all positively correlated. The percentage of TOC was correlated with most of the other indices and at the highest level of probability with BI (0.899). Biodegradability index (BI) was positively correlated with nitrification index (0.739), C:N ratio (0.537) and lignin:cellulose ratio (0.515). Thus, it can be suggested that C:N ratio and BI are most suitable for describing compost maturity. Amongst the stability indices, FDA was found to be highly correlated with CEC/TOC ratio (0.790) followed by organic matter loss percentage (OM loss%) (0.583), ash% (0.541) and DHA (0.509).

Principal component analysis (PCA) reduces the dimension of data matrix and makes an association between variables possible. In this analysis, diagonalization of the correlation matrix takes place and it converts original variables into uncorrelated ones, which represent weighed linear combinations of the original variables known as Principal Components. It provides information for the most important parameters that describe the whole data set. This in turn leads to the reduction of data while maintaining the original information (Alvarenga et al., 2016).

In this study, PCA was run amongst 13 variables of compost stability and maturity, the objective being to assess the similarities and differences between them. The PCA factor loadings revealed significance only for the four principal components based on eigen value > 1 (Table 5). Thus, the original 13 variables could be reduced to four principal components, as they could explain about 86.963% of the total variance. The first component (PC1) explained 43.725% of the variance of the original variables, screened biodegradability index (BI) because of the highest loading (0.920). The other higher loaded factors (OM loss%, ash%, CEC/TOC, FDA, nitrification index, C:N ratio and

Table 4
Pearson's correlation coefficients between maturity and stability indices.

	TOC%	C:N	WSC/Org.N	BI	Nitri.index	Ash%	OM loss%	CEC/TOC	BR Cel.	Lig/Cel.	DHA	FDA	β-Gluco
TOC%	1												
C:N	.516*	1											
WSC/Org.N	.044	.761**	1										
BI	.899**	.537*	.085	1									
Nitri.index	.745**	.508*	.008	.739**	1								
Ash%	-.914**	-.510*	-.036	-.832**	-.626**	1							
OM loss%	-.880**	-.614**	-.162	-.871**	-.611**	.847**	1						
CEC/TOC	-.817**	-.252	.036	-.756**	-.446*	.765**	.730**	1					
BR Cel.	.153	-.157	.075	.133	-.086	-.157	-.023	-.056	1				
Lig/Cel.	.534*	.398	.323	.515*	.315	-.517*	-.499*	-.228	.728**	1			
DHA	-.289	-.079	-.230	-.290	-.065	.281	.155	.415	-.504*	-.566**	1		
FDA	-.594**	-.474*	-.310	-.554**	-.295	.541*	.583**	.790**	.229	-.095	.509*	1	
β-Gluco	.146	.183	-.110	.133	.055	-.061	-.371	.004	-.314	.012	.446*	.061	1

TOC: Total Organic Carbon, WSC: Water Soluble Carbon, Org. N: Organic nitrogen, BI: Biodegradability index, Nitri. index: Nitrification index, OM: Organic matter, CEC: Cation Exchange Capacity, BR: Cellulose biodegradation ratio: Lig: Lignin, Cel:Cellulose, DHA: Dehydrogenase, FDA: Fluorescein Di acetate, β-Gluco: β- Glucosidase.

Values mentioned in bold indicate significant correlation between two parameters. *Correlation is significant at the 0.05 level (2-tailed), **Correlation is significant at the 0.01 level (2-tailed).

Table 5
Factor loadings of each variable along PC1, PC2, PC3 and PC4 which resulted from principal component analysis (PCA).

Indices	PC1	PC2	PC3	PC4
C:N	-.681[a]	-.203	.683[a]	.045
WSC/Org.N	-.301	.202	.904[a]	-.141
BI	-.920[a]	-.101	-.178	.121
Nitri.index	-.688[a]	-.254	-.104	.178
Ash%	.893[a]	.056	.221	-.092
OM loss%	.907[a]	.254	.053	-.181
CEC/TOC	.801[a]	.066	.392	.326
BR Cel.	-.157	.855[a]	-.141	.397
Lig/Cel.	-.610[a]	.572[a]	.128	.490
DHA	.449	-.725[a]	.066	.344
FDA	.711[a]	.108	-.037	.643[a]
β-Gluco	-.084	-.627	.065	.501[a]
Eigen value	5.247	2.217	1.576	1.395
Explained variance (%)	43.725	18.473	13.136	11.629

WSC: Water Soluble Carbon, Org. N: Organic nitrogen, BI: Biodegradability index, Nitri. index: Nitrification index, OM: Organic matter, CEC: Cation Exchange Capacity, BR: Cellulose biodegradation ratio: Lig: Lignin, Cel:Cellulose, DHA: Dehydrogenase, FDA: Fluorescein Di acetate, β-Gluco: β- Glucosidase.
PC1 first principal component, PC2 second principal component, PC3 third principal component, PC4 fourth principal component.
[a] Marked correlations are significant (correlation coefficient >0.5).

lignin/cellulose ratio) were excluded owing to their significant correlations with BI and to reduce redundancy. The second component (PC2) explained 18.473%, while only cellulose biodegradation ratio (BR) was retained after excluding other indices. Similarly, PC3 (13.136%) and PC4 (11.629%) screened WSC/Org. N and FDA as the most sensitive indices (Fig. 3).

3.7. Compost maturity and stability evaluation of parameters

Compost maturity and stability parameters evaluation with national and international standards was done at the end of composting (Table 6). The most important maturity parameter is the C:N ratio which falls in the 15–22 range for different crop residues inoculated with consortia of microbes, whereas in the non-inoculated treatment it ranges from 21 to 46. Nitrification index was in the range of 0.15–0.17 in inoculated treatment. CEC/TOC ratio ranged from 3.27 to 4.18 and biodegradability index was recorded as being between 2.63 and 2.98. Thus, the stability and maturity indices analysed in this study reached the following range (due to different crop residues) of maturity and stability indices

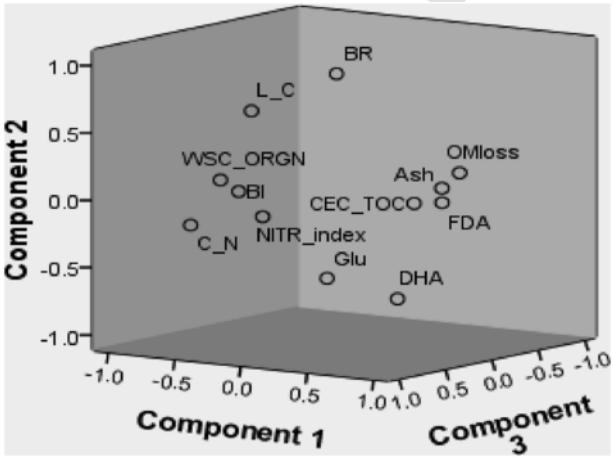

Fig. 3. Biplot of PCA of maturity and stability indices. C_N: C:N ratio, WSC_ORGN: WSC/Org. N, BI:biodegradibility index, NITR_index: Nitrification index, Ash: Ash%, OMloss: Organic matter loss %,CEC_TOC: CEC/TOC, BR: Cellulose biodegradation ratio, L_C: lignin/cellulose ratio.

Table 6
Compost maturity and stability indices comparison with referral values at the final stage of decomposition.

S.No.	Parameters	Observed value		Referral value
		NI	I	
1.	**TOC %**	30–40	19–26	≥16 (FAI, 2007); 25–30 (Awasthi et al., 2014)
2.	**TN %**	0.66–1.19	0.98–1.40	1.0–3.0 (FAI, 2007)
3.	**C:N**	21–46	15–22	<12 (Bernal et al., 1998b); <15 (Bernal et al., 2009b; Morel et al., 1979); 15–20 (Swarnam et al., 2016); <20 (Golueke, 1981; Huang et al., 2006; Manna et al., 2012; FCO, 2013; Zhou et al., 2016); ≤25 (CCME, 2005; TMECC, 2002); <25 (FAI, 2007)
4.	**NH_4-N g/kg**	0.33–0.46	0.32–0.39	<0.4 (Bernal et al., 1998b; Zucconi and Bertoldi, 1987)
5.	**Nitri. index**	0.17–0.24	0.15–0.17	<0.16 (Bernal et al., 1998b)
6.	**WSC %**	0.55–0.86	0.28–0.51	<1.8 (Swarnam et al., 2016); <1.7 (Bernal et al., 1998b); <0.5 (Garcia et al., 1992)
7.	**WSCarb. %**	0.21–0.45	0.06–0.16	<0.1 (Garcia et al., 1992)
8.	**WSC/org.N**	0.65–1.90	0.30–0.70	<0.55 (Antil et al., 2012; Bernal et al., 1998b; Swarnam et al., 2016); <0.7 (Hue and Liu, 1995); <0.3 (Garcia et al., 1992)
9.	**OM % loss**	18.3–39.1	47.3–63.1	>42 (Antil et al., 2012)
10.	**CEC meq/100g**	46–58	72–84	>60 (Harada and Inoko, 1980; Karak et al., 2013)
11.	**CEC/TOC**	1.16–1.92	3.27–4.18	>1.7 (Antil et al., 2012); >3.5 (Garcia et al., 1992)
12.	**BI**	3.20–3.77	2.63–2.98	<2 (Garcia et al., 1992); <2.4 (Morel et al., 1979)

NI: Non-inocululated, I: Inoculated, TOC: Total Organic Carbon, TN: Total Nitrogen, Nitri. index: Nitrification index, WSC: Water Soluble Carbon, WSCarb: Water soluble carbohydrates, Org. N: Organic nitrogen, TOC: Total Organic Carbon: OM: Organic matter, BI: Biodegradability index, CEC: Cation Exchange Capacity.

in the inoculated treatment: TOC (19–26%); TN (0.98–1.40%); C:N ratio (15–22); NH_4-N (0.32–0.39 g kg^{-1}); nitrification index (0.15–0.17); WSC (0.28–0.51%); WSCarb. (0.06–0.16%); WSC/Org. N (0.30–0.70); OM loss% (47.3–63.1); CEC (72–84 meq/100g); CEC/TOC (3.27–4.18) and BI (2.63–2.98). Compost quality assessment was done by comparing the result with the referral values (Table 6).

4. Conclusions

Certain unique conclusions can be made from this study are as follows.

a) The biodegradability index (BI) and C:N ratio emerged as the most suitable for describing compost maturity. For the stability index, FDA was found to be significantly better parameter.
b) Principal component analysis results confirmed that BI, BR, WSC/Org. N and FDA can be regarded as the representative indicators that reflect well the dynamics of rapid compost quality assessment.

The work confirmed that the consortia of fungal inoculants *viz. Tricoderma viride*, *Rhizomucor pusillus*, *Aspergillus awamori* and *Aspergillus flavus* were able to efficiently decompose all the crop residues. To pinpoint, the uniqueness of the present study is that it has assessed sixteen compost quality parameters for seven different substrates at five different stages. Moreover, the results also screened out key indicators of rapid compost quality assessment.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2019.04.015.

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