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RESEARCH ARTICLE

Carbon decomposition by inoculating *Phanerochaete chrysosporium* during drum composting of agricultural waste

V. Sudharsan Varma · Kamma Ramu · Ajay S. Kalamdhad

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Abstract The effect of *Phanerochaete chrysosporium* inoculation during drum composting of agricultural waste was performed at different composting stages. Three trials were carried out with (5:4:1) combination of vegetable waste, cattle manure, and sawdust along with 10 kg of dried leaves with a total mass of 100 kg in a 550 L rotary drum composter. Trial 1 was a control without inoculation of fungus, while trial 2 was inoculated during the initial day (0th day of composting), and trial 3 was inoculated after the thermophilic phase, i.e., on the 8th day of composting period. The inoculation of fungus increased the volatile solids reduction by 1.45-fold in trial 2 and 1.7-fold in trial 3 as compared to trial 1 without any fungal inoculation. Total Kjeldahl Nitrogen (TKN) was observed with 2.31, 2.62, and 2.59 % in trials 1, 2, and 3, respectively, at the end of 20 days of composting period. Hence, it can be concluded that inoculation of white-rot fungus increased the decomposition rate of agricultural waste within shorter time in drum composting. However, inoculation after the thermophilic phase was found more effective than inoculation during initial days of composting for producing more stabilized and nutrient-rich compost.

Keywords *Phanerochaete chrysosporium* · Agricultural waste · Carbon decomposition · Inoculation stage · Rotary drum

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Introduction

Organic waste generated from agricultural activity and urban life poses a major environmental pollution during disposal. Landfilling and open dumping of wastes on the outskirts of towns and cities remain as the primary disposal strategy in India. Globally, India stands the second largest producer of fruits and vegetables and wasting 5.8 to 18 % of the total produced fruits and vegetables (CIPHET 2013). Instead of dumping these organic fractions in open landfills and disposal sites, they can be composted for producing a good quality end product which can be used as a fertilizer for land applications. The successful operation of composting is always followed out by adding several bulking agents such as sawdust, rice straw, dry leaves, and cattle manure to increase the efficiency of process for producing high-quality compost (Huang et al. 2004; Chang and Chen 2010; Varma and Kalamdhad 2014c). Adding these bulking agents in appropriate combinations is reported to provide optimum moisture content, C/N ratio, and pH for the survival of microbes during composting. However, these materials are rich in lignocellulose content contributing to the total organic matter, which is normally resistant for microbial degradation as compared to the readily biodegradable content of organic waste.

There are many reports on the application of rotary drum composter for the processing of organic waste within shorter time period, i.e., 20 days, for various waste materials such as vegetable waste, water hyacinth, and sewage sludge as compared to other composting methodologies (Vuorinen and Saharinen 1997; Kalamdhad et al. 2009; Varma and Kalamdhad 2013; Singh and Kalamdhad 2013; Varma and Kalamdhad 2014b). Most of these reports were experimented on the organic matter transformation, stability, and microbial dynamics during the process. There are not many literatures available on improving the organic matter reduction, i.e., rich in lignocellulose content during drum composting. There are few reports available on the application of white-rot fungi for degradation of agricultural wastes, lignocellulosic fractions, and organic pollutants (Yu et al. 2009; Zeng et al. 2009; Urrea and Reddy 2012). But, there are no reports available on the application of white-rot fungi, i.e., *Phanerochaete chrysosporium* to increase the volatile solids reduction during rotary drum composting of mixed organic waste.

These basidiomycetes belong to the white-rot fungi which are well known for lignocellulose degradation by producing a non-specific extracellular enzyme system consisting of manganese peroxidase, lignin peroxidase, and laccases (Isroi et al. 2011). Hence, the present study was carried out to increase the volatile solids reduction by inoculating *P. chrysosporium* during different stages of rotary drum composting in three different trials. Trial 1 was control without inoculation of fungus, trial 2 was inoculated at 0th day of composting, and trial 3 was inoculated after thermophilic stage, i.e., at 8th day. Finally, all the three trials were correlated for the efficiency of *P. chrysosporium* inoculation during drum composting and its possible effects on the physicochemical and biological parameters of the compost.

Methods

Feedstock materials and processing

Vegetable waste was collected from a vegetable market, Fancy Bazaar, Guwahati, Assam, India, and dry leaves from the Indian Institute of Technology Guwahati campus, Guwahati, India. Cattle manure (buffalo dung) was collected from a dairy farm and sawdust from the nearby Amingaon village. Varma and Kalamdhad (2014c) suggested that mixture of 54 kg vegetable waste, 45 kg cow dung, 9 kg sawdust, and 10 kg of dried leaves was the best combination for producing stabilized compost within shorter time period using rotary drum (20 days). Therefore, the same combinations were experimented for higher carbon decomposition at different inoculation points. Prior to composting, the maximum particle size in the mixed waste was restricted to 1 cm in order to provide better aeration and moisture control. The compost was prepared with three different proportions of vegetable waste, cattle manure, sawdust, and dried leaves. All the waste materials were mixed properly before feeding into the drum. The composition of waste materials is given in Table 1.

Microbial inoculum preparation

The fungus *P. chrysosporium* (MTCC 787) used in this study was procured from IMTECH, Chandigarh, India. The culture was grown on potato dextrose agar (PDA) plates maintained at 25 °C for 12 to 15 days. Ten millilitres of sterile water was poured into 2-week old PDA plates, and the entire fungal growth was scraped off the surface using a sterile rod. The fungal concentration collected in the sterile water was measured and adjusted to the application level of 10^6–10^8 spores g^{-1} of compost to the total mass of 100 kg. After inoculation of spores, the waste materials inside the rotary drum were turned once to spread the inoculum throughout the waste materials (Taccari et al. 2009).

Table 1 Initial combination of waste materials

Waste materials						
Treatment	Vegetable waste (kg)	Cattle manure (kg)	Sawdust (kg)	Dry leaves (kg)	Total (kg)	Fungus inoculation time
Trial 1	45	36	9	10	100	–
Trial 2	45	36	9	10	100	0th
Trial 3	45	36	9	10	100	8th

Drum specifications and analysis

A pilot-scale rotary drum composter of 550 L capacity was operated at batch mode. The drum is of 1.022 m in length and 0.76 m in diameter, fabricated by a 4-mm-thick metal sheet. The composting period of 20 days was decided for both proper degradation and stabilization based on the performance of earlier regarding in-vessel composting reactors. Manual turning was done after every 24 h through one complete rotation of the rotary drum to ensure that the material on the top portion moved to the central portion, where it was subjected to higher temperature based on the performance of earlier studies regarding in-vessel composting reactors (Kalamdhad et al. 2008).

Five-hundred-gram grab samples were collected manually from six different sources without disturbing the adjacent materials. Finally, all the grab samples were mixed thoroughly to make a homogenized sample. Triplicate samples were collected and stored at 4 °C for subsequent analysis. Temperature was monitored using a digital thermometer throughout the composting period. pH and electrical conductivity (EC) of the compost (1:10, *w/v* waste:water extract) were analyzed as described by (Kalamdhad et al. 2009). Volatile solids (VS) were determined by the ignition method (550 °C for 2 h in muffle furnace) (BIS 1982). The total organic carbon was calculated from volatile solids (Mohee et al. 2008). Total Kjeldahl Nitrogen was analyzed using the Kjeldahl method, ammoniacal nitrogen (NH_4-N) using KCl extraction, and total and available phosphorus (acid digest) using the stannous chloride method. Analysis of stability parameters such as CO_2 evolution and oxygen uptake rate (OUR) was performed as described in Kalamdhad et al. (2008). Biodegradable organic matter was measured as soluble biochemical oxygen demand (BOD) (APHA 2005) and soluble chemical oxygen demand (COD) from the supernatant of the blended mixture of 10 g wet sample in 100 mL deionized water. The reported results are the mean of three replicates. The statistical significance of differences between all replicated samples was determined using the SPSS 17.0 system for the three different trials.

Results and discussions

Physicochemical parameters

Changes in temperature, moisture content, pH, and EC

Organic matter degradation during composting is directly indicated by the temperature pattern of the compost, and it can be correlated to the rate at which the degradation is being carried out. Fig. 1 shows the temperature pattern of all the trials over composting period. With higher biodegradable and soluble organic matter in vegetable waste mixture, a fast rise in temperature was observed within few hours of the process in all the three trials. A maximum of 66.4 °C was observed in trial 1 followed by 62.4 and 61.2 °C in trials 2 and 3, respectively. Even though the same combination of waste ratio (5:4:1) was maintained in all the three trials, early thermophilic stage with different temperature patterns was observed towards the end of composting period. This might be due to combination of vegetable wastes and higher indigenous microbial populations and also due to the inoculation of fungus (Varma and Kalamdhad 2014c). The drum was turned once in every 24 h for uniform mixing of waste materials and to attain proper degradation. Even though maximum temperature was observed in trial 1, inoculation of *P. chrysosporium* was observed to increase the overall microbial activity which was evident by the prolonged thermophilic phase in trials 2 and 3. This prolonged thermophilic stage can be attributed to the decomposition of soluble organic matter, and moreover, temperature ranging from 55 to 60 °C is reported optimum for the degradation of lignocellulose content. Hence, with addition of sawdust and dried leaves which are rich in lignocellulose content, prolonged thermophilic phase can be found beneficial for higher degradation.

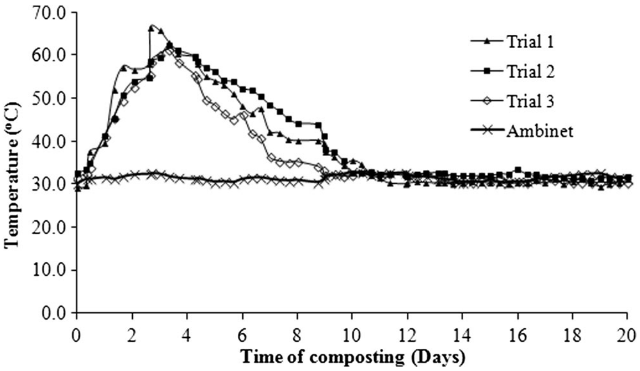

Fig. 1 Temperature variation during composting

Organic matter degradation in composting can be viewed as a result of rise in temperature. As the temperature rises, loss in moisture content can be observed. Figure 3 depicts the loss of moisture content during the composting period. Hence, loss of moisture during the composting process can be viewed as an index of decomposition rate. However, the composting material should have minimum moisture content for the survival of microorganisms (Kalamdhad et al. 2008). With higher temperatures in all the three trials, the moisture loss was found to be 15, 20, and 19 % in trials 1, 2, and 3, respectively. This major reduction is mainly due to the higher and prolonged temperature maintained in the compost. Therefore, it can be considered that higher temperature in the compost environment will lead to major reduction in moisture content. Analyzing the results by ANOVA, the decrease in moisture content varied significantly between the 20 days of composting ($P<0.05$).

Figure 2 shows the variation in pH and electrical conductivity (EC) during the process. The pH of all the three trials increased towards alkaline conditions until the end of composting. The initial pH values were found to be 6.76, 7.18, and 6.54 and finally increased to 7.75, 7.22, and 7.7 in trials 1, 2, and 3, respectively. The degradation of organic nitrogen to the release of ammonia or ammoniacal nitrogen through ammonification can be considered for the increase in

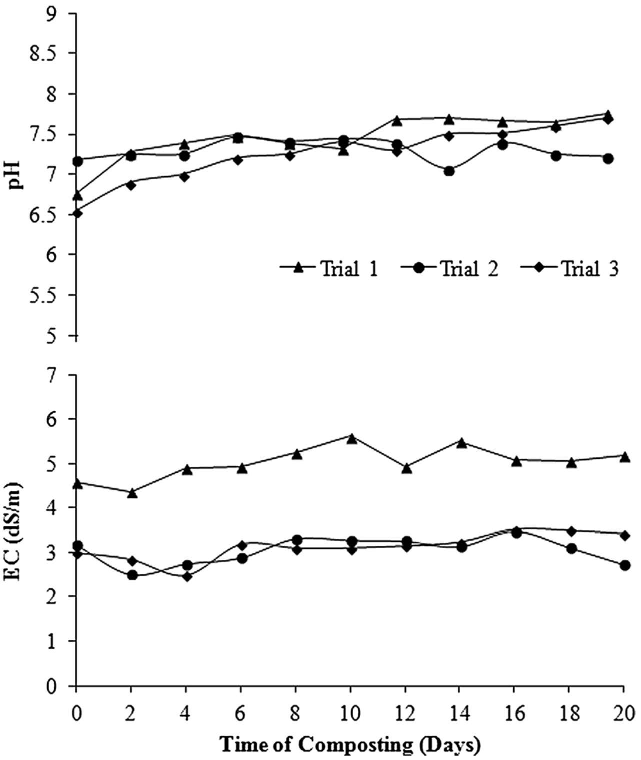

Fig. 2 pH and EC during composting period

pH during composting. Proper aerobic conditions are required for the ammonification, which is maintained by turning the drum once in every 24 h for uniform mixing and release of trapped gasses. With the volatilization of ammonia and precipitation of mineral salts, the EC of the compost could be decreased in the final stages of composting (Wong et al. 1995). Such similar trend was observed during the study in all the three trials. The initial values of the EC were found to be 4.16, 3.18, and 3.00 and finally were observed in the range of 5.18, 2.74, and 3.43 in trials 1, 2, and 3, respectively. Significant difference in pH and EC was observed between all the trials ($P<0.05$).

Carbon decomposition, nitrogen, and phosphorous dynamics

The decomposition of simple and complex organic compounds such as proteins, amino acids, lipids, and sugars will lead to the loss of organic matter as CO_2 evolution and heat. With higher degradation, the total mass of the compost will be gradually decreased, and the loss can be directly viewed by the decrease in total organic carbon (TOC). During composting, the microorganisms use carbon as a source of energy and nitrogen for building cell structure for the degradation of organic matter. The organic matter with excess carbon can utilize more nitrogen leading to robbing of soil nitrogen, while applying compost as soil conditioner. However, inoculation of white-rot fungi leads to higher loss of TOC in the order of 11.4, 16.4, and 19.3 % in trials 1, 2, and 3, respectively (Fig. 3). The higher reduction of TOC in trials 2 and 3 can be considered due to the higher utilization of complex organic matter by the inoculated *P. chrysosporium*. During composting, lignocellulose degradation is considered as key process with the treatment of agricultural wastes. This polymer is difficult to be biodegraded and reduces the availability of lignocellulose to the microorganisms, while the ligninolytic enzymes secreted by white-rot fungi have been widely used to degrade these recalcitrant environmental pollutants (Feng et al. 2011). The added basidiomycetes fungus secretes manganese peroxidase, lignin peroxidase, and laccases enzymes which are majorly involved in the degradation of polymeric compounds, i.e., lignocellulose. Even though the native indigenous microorganisms were able to degrade the organic matter to a maximum of 11.4 %, the added bulking agents were rich in lignocellulose so that higher degradation was not possible.

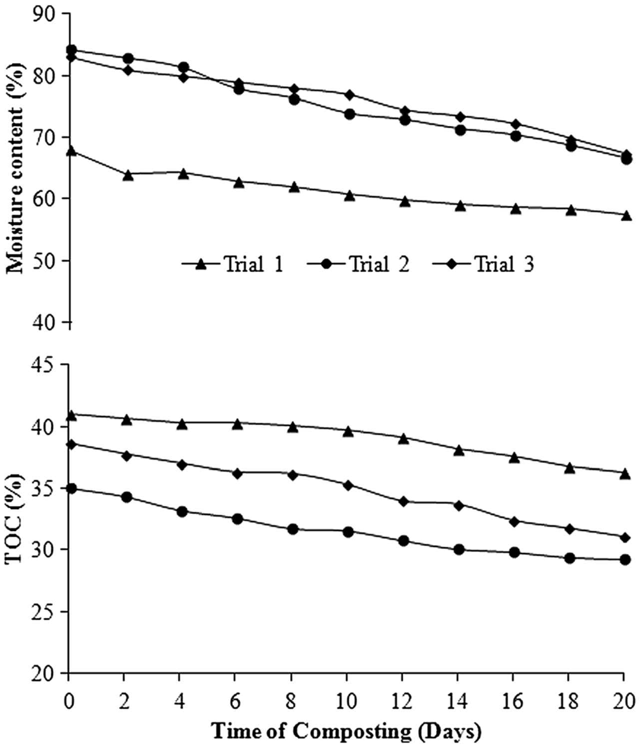

Fig. 3 Moisture and TOC reduction during composting

During the initial period of composting, the soluble and easily degradable carbon sources such as monosaccharides, starch, and lipids are degraded by indigenous microbial population followed by proteins. Finally, the more resistant compounds such as cellulose, hemicellulose, and lignin are degraded and partly transformed into humus (Crawford, 1983). The inoculation of *P. chrysosporium* during the 0 and 8th days in trials 2 and 3 was considered to increase the overall microbial activity thereby achieving higher TOC reduction. *P. chrysosporium* has the optimum survival temperature of 20 to 50 °C as reported by many researchers (Mouchacca 1997; Tuomela et al. 2000). Since the thermophilic phase has reached 60 °C, there are chances of inactivation of the fungi due to such higher temperature. But, in the case of trial 3, where fungus was added after thermophilic phase, the TOC reduction was observed with 19.3 %. The higher reduction of TOC in trial 3 can be considered due to the increased activity of *P. chrysosporium* soon after the thermophilic stage, which was very optimum with the partially degraded lignocellulose content. The higher temperature can be considered to loosen the lignocellulose bonds, making them more available for the microbial degradation after thermophilic stage.

Feng et al. (2011) has reported the increase in carbon decomposition by adding ligninolytic enzymes during the lignocellulosic waste degradation. It was reported that the thermophilic temperature had a major effect in the degradation of hemicellulose and lignin, while cellulose was degraded in the initial phase of composting (Bolta et al. 2003). Such similar results were observed in the present study with higher carbon degradation by inoculating white-rot fungi. Therefore, with the depletion of readily biodegradable organic matter until the end of thermophilic stage, the remaining lignocellulose was degraded further by the *P. chrysosporium* with warm and moist environment. A significant variation in carbon loss was observed between all the trials ($P<0.05$). The population of fungi was in the range of 6.4×10^7 and 5.6×10^8 CFU g^{-1} in trials 2 and 3 during the inoculation. Due to thermophilic temperature, the growth of fungi was affected and was found to be in the range of 2.4×10^3 at the end of composting in trial 2. However, in trial 3, the growth was found to be consistent without any gradual decrease in population of fungi leading to higher loss of carbon as compared to other trials. Finally, at the end of composting, the population of fungi was found to be in the range of 4.6×10^5 in trial 3. The considerable amount of fungi at the end of composting period was in accordance with the reports of Thambirajah et al. (1995) and Varma and Kalamdhad (2014a).

The nitrogen content of the compost mass usually increases with the loss of mass towards the end of composting (Kalamdhad et al. 2008). As the degradation of organic matter was higher with different inoculation stages, the nitrogen was observed to increase in all the three trials. Therefore, the Total Kjeldahl Nitrogen (TKN) was increased from 1.68, 1.41, and 1.57 % to 2.31, 2.62, and 2.59 % in trials 1, 2, and 3, respectively, at the end of 20 days (Fig. 4). The increase in nitrogen can also be attributed by the nitrogen-fixing bacteria in the later stage of composting (Sanchez-Monedero et al. 1999). Higher pH and temperature have been revealed to enhance ammonia loss and decrease NH_4-N during composting (Kalamdhad et al. 2009; Varma and Kalamdhad 2014c). Similarly, NH_4-N was observed to reduce from 0.31, 0.39, and 0.80 % to 0.09, 0.27, and 0.35 % in trials 1, 2, and 3, respectively. Decrease in NH_4-N can also be considered due to immobilization as nitrogenous compounds such as amino acids, nucleic acids, and proteins by microbes (Sanchez-Monedero et al. 1999). Significant difference in the loss of ammoniacal nitrogen was observed in all the trials ($P<0.05$).

The mineralization of inorganic phosphate from organic phosphate is majorly dependent on the temperature and moisture of the compost environment. The process is more rapid in warm and moist conditions which was observed during the process. Inorganic phosphorus is negatively charged, reacts readily with positively charged iron (Fe), aluminum (Al), and calcium (Ca) ions to form relatively insoluble substances (Bauer A and Velde 2014). When this occurs, the phosphorus is considered fixed. Higher amounts of total and available phosphorous (TP and AP) during the initial period of composting can be due to the mix of waste materials in different combinations (Fig. 5). Due to higher degradation and elevated temperature, the TP and AP values of the compost has increased during the process. TP was observed to increase from 3.41, 3.26, and 4.01 g kg^{-1} to 4.30, 4.96, and 4.54 g kg^{-1} in trials 1, 2, and 3, respectively. Similarly, AP was observed to increase from 1.41., 1.85, and 2.06 g kg^{-1} to 1.49, 2.72, and 2.5 g kg^{-1} in trials 1, 2, and 3, respectively, at the end of 20 days (Fig. 4). The higher increase in TP and AP during the thermophilic stage was supported by (Varma and Kalamdhad 2014c).

Biological parameters

During composting, large organic molecules are broken down to smaller and soluble ones by the action of microbial communities. During this process, higher OUR is observed in initial period and the final stages with lower OUR values due to the deprival of readily available organic matter (Iannotti et al. 1993; Varma and Kalamdhad 2014a). The reduction in

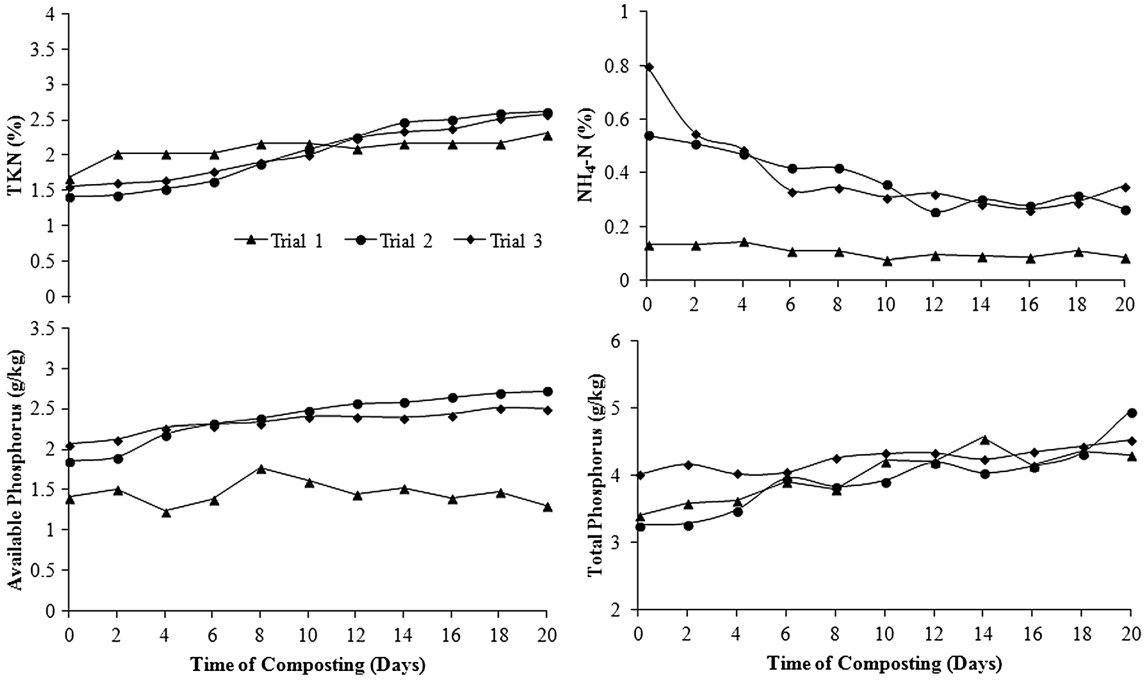

Fig. 4 Nitrogen and phosphorous dynamics during composting

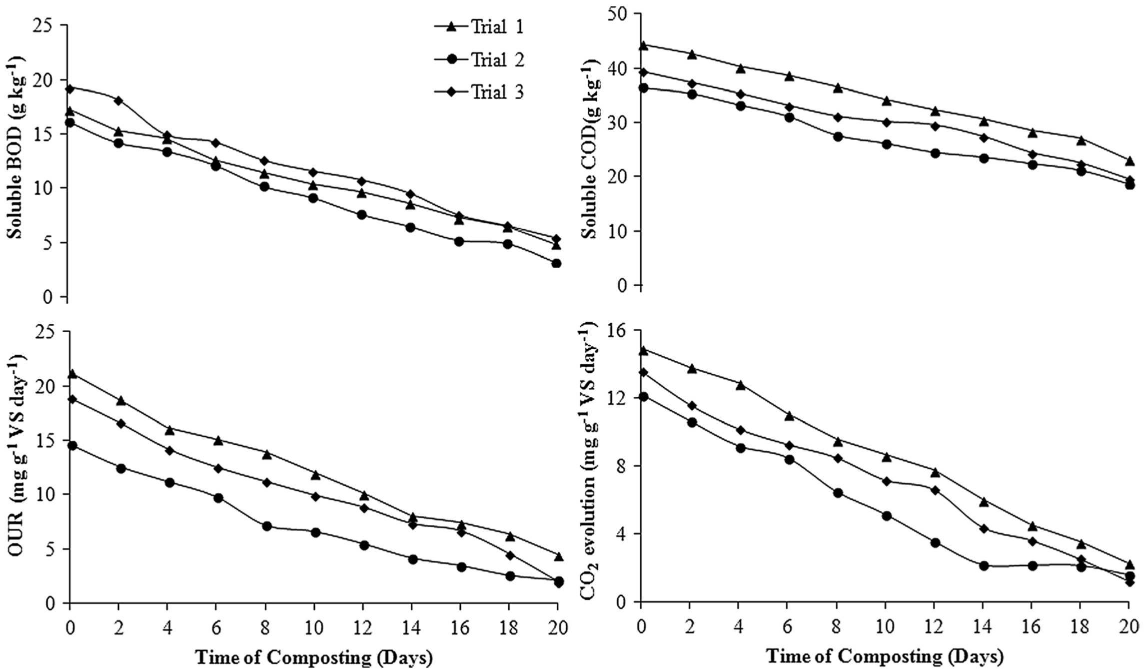

Fig. 5 sBOD, sCOD, OUR, and CO_2 evolution during composting

OUR and CO_2 evolution values during the process can be considered due to degradation process (Singh et al. 2013). Hence, with higher degradation, the OUR decreased from initial values of 21.26, 14.65, and 18.87 mg g^{-1} VS day^{-1} to 4.49, 2.21, and 1.98 mg g^{-1} VS day^{-1}, in trials 1, 2, and 3, respectively. Similarly, CO_2 evolution values decreased from 14.97, 12.24, and 13.64 mg g^{-1} VS day^{-1} to 2.28, 1.64, and 1.24 mg g^{-1} VS day^{-1} in trials 1, 2, and 3, respectively, at the end of 20 days (Fig. 5). The lower OUR and CO_2 evolution at the end of composting period denotes the stability of compost and similar such values were observed during the process within 20 days (Kalamdhad et al. 2008; Varma and Kalamdhad 2014a). Significant differences in the oxygen uptake and CO_2 evolution rate were observed in all the trials ($P<0.05$).

The organic fractions in the compost mix can be directly measured as soluble BOD and COD. The percentage of the readily bioavailable organics has been considered important for the compost quality (Bernal et al. 1997). The organic fraction degradation can be measured by the decrease in soluble BOD and COD. With proper mixing and agitation,

Table 2 Micronutrient and heavy metals during composting period

Day	Na (g kg^{-1})			K (g kg^{-1})			Ca (g kg^{-1})		
	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
0	1.25±0.14	3.4±0.08	2.43±0	14.08±0.4	35.59±0.05	22.44±0.77	8.27±0.8	8.88±0.06	8.49±0.3
20	1.36±0.04	2.67±0	3.29±0.04	25.67±0.67	36.38±0.03	31.82±1.08	10.36±0.94	10.41±0	20.79±0.16
Day	Cd (mg kg^{-1})			Cr (mg kg^{-1})			Cu (mg kg^{-1})		
	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
0	70.25±1.06	57.5±0.71	55.5±1.06	26.5±0.71	15.5±1.77	5±0.71	49±0	40.5±5.66	34.5±0
20	75±1.41	54.5±0.71	56±1.41	177.5±3.54	17±1.41	6.5±3.54	549.5±0.63	78.5±1.06	25±637.1
Day	Mn (mg kg^{-1})			Ni (mg kg^{-1})			Pb (mg kg^{-1})		
	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
0	673.5±4.95	549.5±14.85	712.5±4.95	215.75±3.89	290.5±0.31	272±0.32	855±21.21	1035±28.28	984.5±21.21
20	865±5.66	550±9.55	802.5±5.66	41±7.07	277.5±0.19	274.5±0.25	892.5±38.89	989.5±31.82	990±38.89
Day	Mg (g kg^{-1})			Fe (mg kg^{-1})			Zn (mg kg^{-1})		
	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
0	5.10±0.21	8.03±0.17	5.55±0.14	6914±36.77	662.5±0.45	3302.5±0.37	262.9±0.14	160±1.41	141±0.14
20	6.99±0.33	10.44±0.06	6.34±0.05	10782±11.31	708.5±0.38	4583.5±0.38	353.1±4.45	150.5±0.67	152.5±4.45

higher degradation was carried out during the process by which the soluble BOD and COD are decreased drastically, resulting in decreased emission of carbon dioxide, ultimately indicating the stabilization of compost. Soluble BOD values decreased from 17.35 to 4.92 g kg^{-1} in trial 1, 19.36 to 5.51 g kg^{-1} in trial 2, and 16.24 to 3.25 g kg^{-1} in trial 3 within 20 days of composting period. Correspondingly, soluble COD values decreased from 44.41 to 23.21 g kg^{-1} in trial 1, 36.45 to 18.65 g kg^{-1} in trial 2, and 39.45 to 19.65 g kg^{-1} in trial 3 (Fig. 5). Significant differences in the loss of soluble COD and BOD were observed in all the trials ($P<0.05$).

Micronutrient and heavy metals

Table 2 illustrates the increase in total concentration of micronutrients (Na, K, Ca, Mg) and heavy metals (Cr, Cd, Ni, Pb, Fe, Mn, Zn, and Cu) in trials 1, 2, and 3 during the 20 days of composting period. During composting, micronutrients and heavy metals are observed to increase due to mass loss caused by the mineralization of organic fractions, and they are found in significant amounts in vegetable waste and cattle manure (Fang and Wong 1999).

Conclusion

Inoculation of white-rot fungus for decomposition of agricultural waste was found effective in terms of higher organic matter degradation and nutrient-rich compost. Application of rotary drum composter for agricultural waste composting was found beneficial in providing uniform mixing and proper aeration for higher decomposition within shorter time, i.e., 20 days of composting period. A maximum of 19.3 % reduction of TOC was found in trial 3 as compared to 16.4 and 11.4 % in trials 2 and 1, respectively. In addition to higher degradation, the final compost of trial 3 was observed in 2.59 % and 4.54 g kg^{-1} of TKN and TP, respectively. Since, the thermophilic phase had effect on the growth of *P. chrysosporium*, inoculation after the thermophilic phase could be beneficial for higher degradation of organic matter. Finally, it can be concluded that inoculation of fungal concentration at the level of 10^6–10^8 spores g^{-1} of compost after thermophilic stage would be effective during rotary drum composting of agricultural waste.

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