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Bacterial and Fungal Biodegraders Consortia for Effective Decomposition of Wheat Straw to Obtain Nutritive Organic Compost

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Wheat straw is an abundant by-product from wheat production. The average yield of wheat straw is 1.3-1.4 lb per lb of wheat grains. Composting is a cost effective and eco-friendly process to dispose abundant agricultural wastes. In our study we apply bacterial and fungal biodegrader consortia to enhance quality of compost. The parameters like pH, moisture, cellulose, lignin, TOC, total nitrogen, C: N ratio and lignocellulolytic enzymatic properties have been analyzed. The C: N ratio at 90 days was 15.02% with mixing of bacterial consortium and 17.04% with treatment receiving fungal consortium as compared to natural composting which were 25.30%. Endocellulase activity increases up to 60 days that was 93.10 IU/g in treatment receiving bacterial consortium while in other lignin peroxidase and laccase was detected in very less amount. The data suggested that bacterial and fungal mixture can degrade wheat straw maximum between 60-90 days.

Key words: Compost, Enzymatic profile, Physico chemical analysis.

Wheat is a staple food for 2.45 billion people (35 percent of the world's population) and about 30 million people are engaged in wheat cultivation (Lumpkin, 2011). For every 1.3 kg of wheat grain produced, about 1 kg of straw is produced (Ruiz *et al*., 2012). This resulted in about 534.23 million tonnes of wheat straw in 2011. However, most of the straws are burnt in the field which causes significant environmental and health problems as well as traffic accidents in addition to loss of a valuable resource (Mittal *et al*., 2009; Yang *et al*., 2008).

Wheat straw is a lignocellulosic material that contains cellulose, hemicellulose and lignin as major components. In nature, strains of fungi and bacteria, including species of genera *Aspergillus* (Emtiazi *et al*., 2001); *Trichoderma* sp. (Perez *et al*., 2002); *Cyathus stercoreus* (Keller *et al*., 2003); *Lentinus edodes* (Songulashvili *et al*., 2005; Brienzo *et al*., 2007); *Trametes pubescens* (Melamane *et al*., 2007); *Pleurotus* sp. (Ragunathan and Swaminathan, 2004; Mukherjee and Nandi, 2004; Belewu, 2006; Locci *et al*., 2008); *Penicillium camemberti* (Taseli, 2008), *Phanerochaete chrysosporium* (Das and Hossain, 2000; Shi *et al*.,) and cellulolytic bacteria such as *Clostridium thermocellum*, *Streptomyces* sp., *Ruminococcus* sp., *Pseudomonas* sp., *Cellulomonas* sp., *Bacillus* sp., *Serratia*, *Proteus*, *Staphylococcus* sp. and *Bacillus subtilis* (Wood and Bhat,1988) have been studied from the point of understanding the enzyme systems involved in cellulose degradation.

Synergistic action of cellulolytic and lignolytic enzymes result in the degradation of complex organic lignocellulosic transformation into stable organic matter containing humic-like

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compounds. These substances positively affect soil ecology, structure, fertility and productivity and considered a critical factor for agricultural production. The promising cellulolytic and lignolytic microorganisms can be employed for production of lignocellulolytic enzymes viz., endo- and exo-cellulases, ^{2}-glucosidase, hemicellulases, lignin peroxidases, manganese peroxidases and laccases by using different agro-wastes as carbon source during solid bioconversion process.

The objective of present research is organic waste transformation in to preeminent compost material by bacterial and fungal community and to reduce long time required for maturity of compost.

MATERIALS AND METHODS

Microorganisms and maintenance

The bacterial cultures *viz. Cellulomonas cartae*, *Pseudomonas putida*, *Pseudomonas fluorescens*, *Lactobacilli plantarum*, *Bacillus megaterium*, *Bacillus subtilis* and the fungal cultures *viz. Chaetomium globosum*, *Pleurotus ostreatus*, *Coriolus versicolor*, *Trichoderma viride*, *Trichoderma harzianum*, *Emericella nidulans*, *Aspergillus niger*, *Aspergillus wentii* and *Aspergillus terrus* were made available from Department of Microbiology, A.A.U., Anand for the study.

Bacterial and fungal cultures were maintained on Nutrient agar medium and Potato dextrose agar respectively for routine use. For long term storage, the bacterial and fungal cultures were maintained on N agar and PDA slants and covered with glycerol after proper growth respectively and stored at 0 - 4° C.

Formulation of bacterial and fungal biodegrader consortia

An important prerequisite for successful development of microbial culture mixtures depend on the compatibility (tolerance) of co inoculated microorganisms. *In vitro* plate bioassay was carried out to determine compatibility of bacterial culture on N agar plate and fungal cultures were cross streaked each other on PDA plate by dual culture method.

Effective bacterial cultures able to degrade cellulose and lignin individually were converted in consortium formulation as per the method proposed by Sarkar *et al.*, 2011. The bacterial count of the consortium was kept 3.4×10^{9} per ml.

Fungal consortium based on individual fungal members was prepared following method proposed by Naik, 2007. The initial population in the fungal consortium was kept 3.2×10^{9} spores/ml.

Compost material

Wheat straw was used as experimental materials collected locally from wheat farm Lambhvel village of Anand District of Gujarat State, India. Visual evaluation deemed the straw to be of good quality with no apparent signs of mould or decay. Immediately after transport to laboratory, the materials were analyzed for all physicochemical parameters as mentioned in Table 1. The straw was cut to the 3-5 cm size in length and dried in oven at 50 °C till it keeps a constant weight.

Experimental design

A net house experiment was conducted using plastic containers (crates) to determine efficiency of bacterial and fungal consortia degrade wheat straw.

Five kilograms of uniform size clean wheat straw and 500 grams of sterile soil were mixed together and amended with R.O. water to obtain a moisture content of about 60% (w/w). The substrate container were inoculated with 10% (v/w) of respective microbial consortium having concentration 10^{9} cell ml^{-1} and incubated at near natural condition in net house up to 90 days. Moisture level in the compost was maintained by sprinkling R.O. water once in two days. The substrates were turned twice in a week. Sample was taken for physical, microbial and enzyme activities on 0, 30, 60 and 90 days after inoculation.

Measurement physico-chemical parameters

pH at every 30 days interval up to 90 days were measured by standard method of potentiometer and moisture content was determined by dry weight in oven at 105°C for 24 h.

Estimation of reducing sugar and lignocellulolytic enzymes

Reducing sugar content was estimated at every 30 days interval by DNSA method as described by Miller (1959). Five g of sample was withdrawn from crates and mixed with 20 ml of cold 0.05 M acetate buffer (pH 6.5). The homogenate

was filtered through nylon cloth of 200 meshes and the filtrate was centrifuged at 8000 rpm at 4 °C for 20 minutes. The su-pernatant was analyzed for enzyme activities *viz.* carboxy methyl cellulase (CMCase), filter paper activity (FPase), ² - glucosidase, laccase and lignin peroxidase activity.

The activity of cellulases (FPU, CMCase and cellobiase) was estimated in the culture filtrates after 30 days in solid state bioconversion of wheat straw by the method described by Ghose *et al*. (1983). Laccase activity was determined by the oxidation of 2, 2'–azino–bis (3-ethylbenzethiazoline-6-sulfonate), ABTS at 37°C as per the method proposed by Buswell and Odier (1987). Lignin peroxidase (LiP) activity was determined by the method proposed by Tien and Krik (1988) which involved oxidation of veratryl alcohol to vetraldehyde at 37°C as indicated by an increase in $A_{310.}$

Estimation of cellulose and lignin content

Cellulose content in samples was measured by Anthrone assay method proposed by Updegraff (1969). Lignin content was measured by the gravimetric method proposed by Chesson (1978).

Estimation of organic carbon and total nitrogen

To determine amount of organic carbon method was proposed by Walkley and Black (1934). Nitrogen in the test samples was estimated by the Micro Kjeldhal method as described by Jackson (1973). The C: N ratio was calculated by dividing per cent of organic carbon by per cent total nitrogen.

FTIR analysis (Fourier Transform Infrared Spectroscopy) of treated and untreated wheat straw

Fourier Transform Infrared Spectroscopy (FTIR) works by exciting chemical bonds with infrared light and is best for identification of organic materials. The different chemical bonds in this excited state absorb the light energy at frequencies unique to the various bonds. This activity is represented as a spectrum. The spectrum can be expressed as % transmittance (%T) or % absorbance (%A) versus wave number.

The FTIR spectra were considered to examine the functional group changes occurred due to the cellulolytic degradation of wheat straw material used. FTIR spectra were recorded by the FTIR spectrometer. The FTIR analysis was performed on the microbial degraded wheat straw (after 30 days of incubation), being compared with control material. The cellulolytic degraded wheat straw was collected and washed with distilled water for removal of bacterial cells and fungal mycelium bound enzymes and reducing sugars and then dried overnight in an oven at 60°C. The dried samples were embedded in diamond attenuated total reflectance FTIR spectroscopy using a Nicolet Magna-IR 550 FTIR spectrometer (Thermo Electron, Warwick, U.K.) fitted with a potassium bromide beam splitter and a deutroglycine sulphate detector. The FTIR spectra were recorded in the absorption band mode in the range of 4000 – 400 cm^{-1} with a resolution of 4 cm^{-1} and 32 scans.

RESULTS AND DISCUSSION

All the bacterial cultures were found compatible with each other. The compatibility of fungal biodegrader cultures were checked by dual culture method on PDA *in vitro*. All the fungal isolates were found to be coexisted and complimentary with each other. All the fungi were growing without interference and no antagonistic effect was seen (Figure 1). Fungal and bacterial members were used to prepared unique consortium and used in various composting experiments.

For preparation of bacterial consortium individual bacterial cultures were grown in Mendel's medium and mixed together for development bacterial biodegrader consortium with initial population of 6.4×10^9 (cfu/ml) and stored at room temperature in laboratory.

For the preparation of fungal consortium all cultures were tested for compatibility and individual fungal spores inoculated into Mendel's mineral salt broth pH 6 and incubated on gyro rotary shaker at 200 rpm till spore count obtained up to 10^9 spores / ml.

pH and moisture content

pH is an important parameter in composting. The concentration of hydrogen and hydroxyl ion had a definite effect on the lignocellulosic enzyme activity and efficiency of bacterial and fungal consortium. The pattern of changes of acidity or pH in the composting process is presented in figure 1.

Results show that the material becomes slightly acidic at the start of composting as the

initial products of breakdown are the simple organic acids. pH at initial stage remain acidic but after 30 days pH was slightly increased and it was nearly 7 to 7.6 which is favourable range for the growth of bacteria and fungi inoculated in the consortium. The soluble and easily degradable carbon sources utilised by microorganism in the early stage of composting, resulting the pH decreases. Organic acids are formed from these compounds during bacterial and fungal degradation of wheat straw

Table 1. Physicochemical parameters of the wheat straw

S. No	Parameters	Wheat straw	Method
1	pH	6.89	Potentiometer (Jackson, 1973)
2	EC(µs/m)	9.75	Conductivity meter(Jackson, 1973)
3	Moisture content (%)	8.64	AOAC,1980
4	Organic carbon (%)	41	Datta *et al*.,1962
5	Total nitrogen (%)	0.97	Datta *et al*.,1962
6	Total Phosphorous(%)	0.14	Olsen's (0.5 M $NaHCo_{3,}$ pH 8.5) method. (Olsen *et al.*, 1954)
7	Total potassium (%)	1.44	Flame photometry (Neutral N-NH_4OAc) method (Jackson, 1973)
8	Cellulose Wt (%)	32.9	Anthrone method (Updegraff, 1969)
9	Hemicellulose wt (%)	24	NDF method (Georing and Vansoest,1975)
10	Lignin wt (%)	8.9	gravimetric method (Chesson, 1978)
11	Ash wt (%)	7.4	gravimetric method (Chesson, 1978)

Table 2. Physicochemical parameters of compost prepared by using consortium

parameters	30 day			60 day			90 day		
	Control (WS+SS)	WS+SS+BC	WS+SS+FC	Control (WS+SS)	WS+SS+BC	WS+SS+FC	Control (WS+SS)	WS+SS+BC	WS+SS+FC
pH	6.25	6.34	6.20	6.73	6.75	6.44	6.95	7.00	6.72
Moisture	52	60	48	50	52	55	55	50	45
Cellulose (%)	30.70	26.95	28.85	26.12	21.09	25.38	24.07	18.01	19.45
Lignin (%)	7.71	7.66	7.60	6.97	5.47	6.49	6.10	4.33	5.48
Total C (%)	38.16	34.23	36.12	35.89	28.10	30.82	36.12	30.82	24.86
Total N (%)	1.13	1.22	1.15	1.23	1.40	1.26	1.34	1.55	1.46
C:N ratio	34.13	28.17	31.85	29.10	20.08	24.39	25.30	15.02	17.04

WS+SS =Wheat straw +500 g sterile soil,
WS+SS+BC = Wheat straw +500 g sterile soil+ Bacterial consortium,
WS+SS+FC = Wheat straw +500 g sterile soil+ Fungal consortium

Table 3.

parameters	30 day			60 day			90 day		
	Control (WS+SS)	WS+SS+BC	WS+SS+FC	Control (WS+SS)	WS+SS+BC	WS+SS+FC	Control (WS+SS)	WS+SS+BC	WS+SS+FC
Endocellulase(U/g)	1.30	78.37	65.21	1.43	93.10	80.88	1.47	79.15	65.66
â -glucosidase(U/g)	1.15	21.29	12.67	1.38	34.66	23.69	1.28	22.75	17.65
Filter paper(U/g)	1.20	17.05	10.62	1.26	30.92	20.84	1.20	22.72	10.87
Lignin peroxidase (U/g)	0.73	1.63	1.52	0.75	1.93	1.84	0.62	0.79	0.78
Laccase(U/g)	0.01	0.33	0.27	0.01	0.27	0.31	0.07	0.17	0.21

which has influence the pH. After 45 days of inoculation bacterial and fungal biodegrader consortium starts degrading proteins, resulting in the liberated ammonium and an increased the pH.

Figure 2 shows changes in moisture content during decomposition of wheat straw by biodegrader consortium. Moisture content has been referred to as a critical factor in optimizing composting systems48, 49, because the decomposition of organic matter depends on presence of water to support microbial activity. Initial moisture content for the treatment receiving bacterial biodegrader consortium was 55 % for wheat straw. At 90 DAI moisture content was maintained above 50 % the favourable range during entire composting period. Similarly in treatments receiving fungal biodegrader consortium the initial moisture content was 56 % for wheat straw.

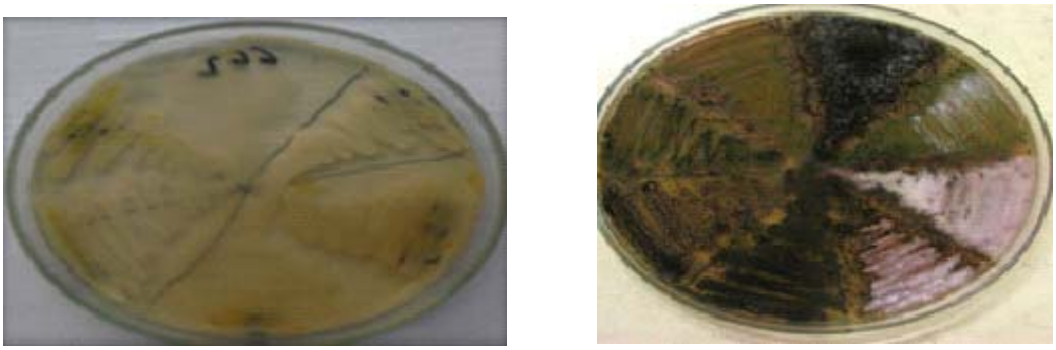


Fig. 1. *In vitro* compability of bacterial and fungal cultures

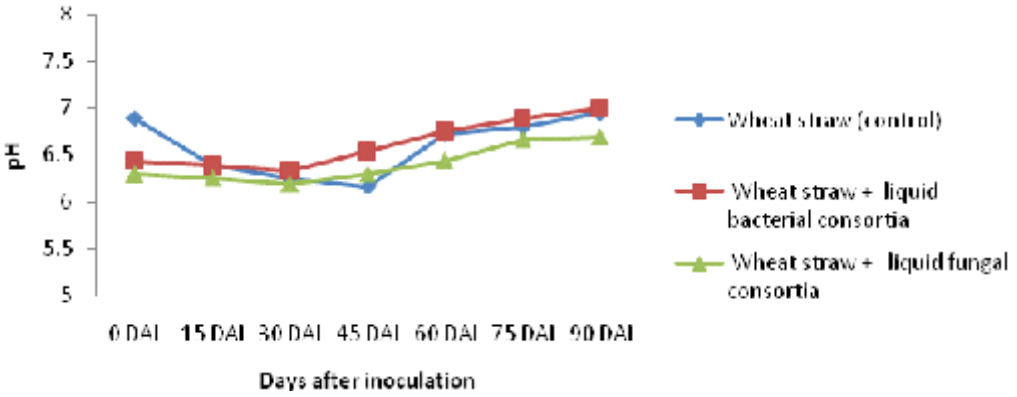


Fig. 2. pH change during the biodegradation of wheat straw

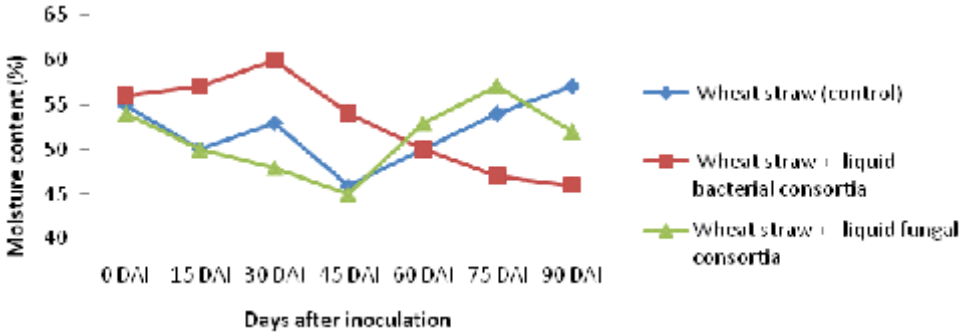
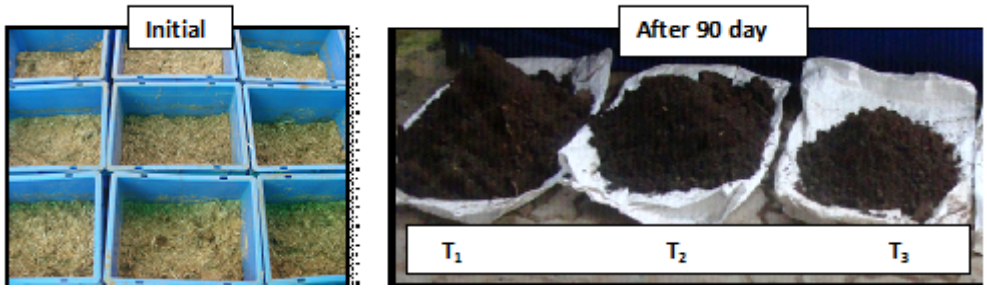


Fig. 3. Moisture content of wheat straw during biodegradation

Moisture content below 40 % can decline microbial activity considerably Initial moisture content around 60% provides good condition for degradation whereas in prepared or mature compost, 10-15% moisture should be maintained for retaining the microbial population of compost. Consequently, high moisture content must be avoided because water displaces air from the interstices between particles and creates anaerobic conditions. Very low moisture content may deprive the organisms of water needed for their metabolism and inhibit their activity.



Treatment details

T_1 Wheat straw + 500 g sterile soil

T_2 Wheat straw + 500 g sterile soil +bacterial consortia

T_3 Wheat straw + 500 g sterile soil + fungal consortia

Fig. 4. Biodegradation of the wheat straw in crates

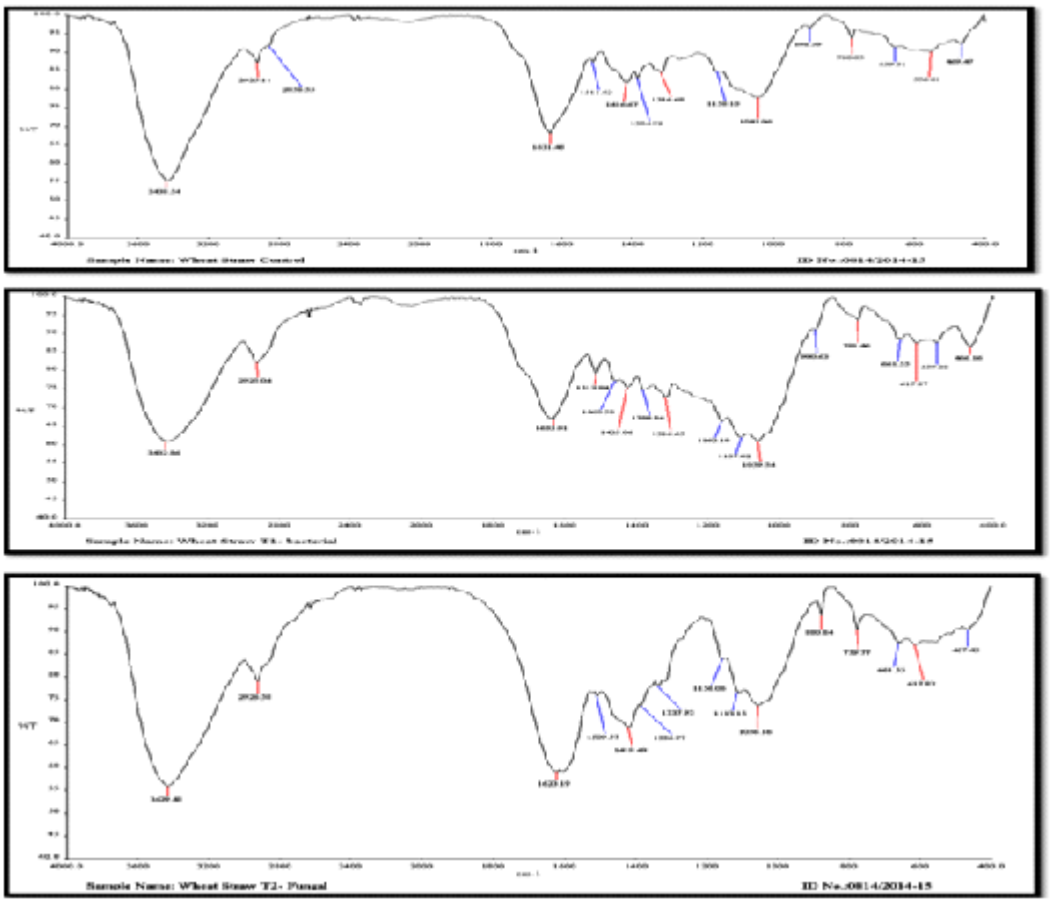


Fig. 5. FTIR analysis of treated and untreated wheat straw

Singh (2011) reported that the loss of organic matter increased as moisture content up to 70 per cent and also decreased the rate of decomposition. Inbar *et al.*, 2013 observed that that moisture content had a major effect on oxygen consumption. They reported that the oxygen consumption was higher at higher moisture content and microbial activity declined.

Cellulose and lignin content

Wheat straw mixed with sterile soil and biodegrader consortium showed cellulose and lignin content which was found significantly low as compared to control which does not received any biodegrader consortium application.

Organic carbon, nitrogen and C/N ratio

Organic matter is mineralized after composting, mostly due to degradation of easily degradable compounds, which are utilized by microorganisms as carbon and nitrogen sources. Rate of organic matter loss is an indicator of the overall composting rate. While degrading organic compounds, microorganisms convert 60 to 70% carbon to carbon dioxide and remain 30-40% into their bodies as cellular components. In our study treatments receiving wheat straw mixed with sterile soil and application of consortium showed organic carbon content of 30.82 and 24.86% which was lower as compared to control 36.12%. Treatments received with bacterial and fungal consortium in wheat straw mixed with sterile soil showed total nitrogen 1.55 and 1.46 % which was superior to control (1.34 %). Wheat straw mixed with sterile soil and bacterial consortium showed C: N ratio of 15.02 and fungal consortium 17.04 % which was significantly low compared to control (25.30 %). The phenomenon of C: N ratio of fully degraded material and mature compost should be >20 % (Hamoda *et al*, 1998) is matches with present C: N trends by biodegrader consortium in crates.

Characterizing and quantifying the enzymatic activity during composting can reflects dynamics of the composting process in terms of the decomposition of organic matter and nitrogen transformation and may provide information about the maturity of composted product (Tiquia,2002).

Treatments receiving bacterial consortium showed highest endocellulase (93.10 U/g), ^{2} glucosidase (34.63 U/g) and their filter paper activity (30.92 U/g) at 60 DAI. Wherein, treatments receiving fungal consortium showed highest endocellulase (80.88 U/g), ^{2} glucosidase (23.69 U/g) and corresponding filter paper activity (20.84 U/g) at 60 DAI.

Lignin degrading enzymes *viz.* Laccase and lignin peroxidase were detected very less amounts in composting process going on.

FTIR analysis

FTIR spectra of uninoculated wheat straw waste are shown in figure 3. A strong hydrogen bonded (O–H) stretching absorption is seen at 3438 $cm^{"1}$ (1) and a prominent C–H stretching absorption around 2925-2856 cm^{-1}. In addition, there are many well-defined peaks in the fingerprint region between 1800 and 800 $cm^{"1}$. The peaks in the fingerprint are assigned 1631 $cm^{"1}$ for absorbed O-H and conjugated C-O,1511 $cm^{"1}$ for aromatic skeletal of lignin, 1416 $cm^{"1}$ for C-H deformation of lignin and carbohydrates, 1384 $cm^{"1}$ for C–H deformation in cellulose and hemicellulose, 1316 $cm^{"1}$ for C–H vibration in cellulose and Cl–O vibration of syringyl derivatives, 1158 $cm^{"1}$ C-O-C vibration of cellulose and hemicellulose, 1035 $cm^{"1}$ for C–O stretch of cellulose and hemicellulose and 898 $cm^{"1}$ for C–H deformation of cellulose.In wheat straw treated with bacterial as well as fungal biodegrader consortium peak 1416 cm"1 for C-H deformation in lignin and carbohydrates was removed at 90 DAI.

Waghmare *et al.* (2014) reported that in the grass powder 1725 cm-1 for unconjugated C-O stretch (hemicelluloses), 1508 cm-1 for aromatic skeletal vibration (lignin) are removed, 1161 cm-1 for C-O-C vibration (cellulose and hemicelluloses) are also removed after microbial enzymatic degradation. While in sorghum husks substrate, the absorbance peak at 1736 cm-1 for unconjugated C-O stretch (hemicelluloses) and 1508 cm-1 for aromatic skeletal vibration (lignin) are removed after the microbial enzymatic degradation.

CONCLUSION

Overall results revealed that bacterial consortium comprising of *C. cartae*, *P. putida*, *P. fluorescens*, *L. plantarum*, *B. megaterium*, *B. subtilis* and fungal consortium comprising of *C. globosum*, *P. ostreatus*, *C. versicolor*, *T. viride*, *T. harzianum*, *E. nidulans*, *A. niger*, *A. wentii*, *A. terrus* showed viability up to 12 months. The developed formulation showed production of cellulose and lignin degrading enzymes *viz*.

carboxy methyl cellulase (CMCase), filter paper activity (FPase), ² - glucosidase, laccase and lignin peroxidase useful for degradation of wastes which are also indicative in FTIR analysis result.

Application of consortium in net house showed rapid increase in bacterial and fungal population on the agro waste material which have fasten the process of biodegradation to achieve nutritionally rich compost with low C: N ratio compared to control but lab to land at big way is needed. Moreover, all the physicochemical parameters measured for support bacterial and fungal consortiums to be for farmer's convenience and for nurturing soil ecosystem with best nutritive organic compost.

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