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Research Article

INFLUENCE OF MICROBIAL INOCULANTS AND THEIR ENZYME COMPLEXES ON WINDROW COMPOSTING OF PRESSMUD SPRAYED WITH TREATED DISTILLERY SPENTWASH

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ABSTRACT

The study attempted using single, dual and triple inoculant treatments on windrow composting of pressmud treatment (T7) performed better in exhibiting better extracellular enzyme complexes such as cellulose, pectinases and xylanases compared to dual and single and there by exerting early maturity of the pressmud compost within 45 days. Changes in pH, EC and C:N ratio, were determined during windrow composting of pressmud sprayed 90 day. The triple inoculant treatment attained its stability in pH 7.1, EC 2.9dsm-1 and C:N ratio 21:1 with in 45days. Whereas dual required an addition of 15 days and single required an addition of 30 days. The mesophilic and thermophilic microbial inoculants in triple inoculant mixer were steadily found throughout the period of composting enabling an efficient extracellular enzyme complex.

INTRODUCTION

Spread composting is method of solid waste management where by the organic component of solid waste is biological decomposed and stabilized under controlled conditions to a state where it can be handled, stored and or applied to the land without adversely affecting environment. The goals of composting range from sanitation, reducing offensive odours and volume of the waste to inactivating pathogens, parasites and weed seeds and sterilizing the organic constituents for producing a uniform organic material highly suitable for soil application (Gotass 1956).

Generally, successful composting depends on a number of factors that have both direct and indirect influence on the activities of the microorganisms. They include the type of raw materials being composted, its nutrient composting, moisture content, temperature, acidity or alkalinity and aeration. Large quantities of pressmud @ 3% of cane crushed, is produced during sugar manufacture. It is a soft, spongy, amorphous and dark brown to brownish material, which contains sugar, fibre and coagulated colloids, including cane wax, albuminoids, inorganic salts and soil particles (Gupta, antil and Jagnath, 1978). The composting of pressmud varies depending upon the quality of cane and process of cane juice clarification followed. The benefit of pressmud as a source of nutrients and organic amendment for the reclamation of sodic soils has been well established (Gupta and Abrol, 1990). But fresh pressmud has vides C:N ratio and evolves a lot of heat during decomposition. Hence, it should be applied only after proper decomposition. Pressmud composting can be done by mixing with distillery effluent which is also a rich source of nutrients. The composting process was brought about by several organisms such as bacteria, fungi, actinomycetes and protozoa and may also involve invertebrates such as nematodes, potworms, earth worm, mites and various other organisms (Mac Donald *et al.*, 1981). However, the sole agents of decomposition of carbonaceous materials are the heterotrophic microorganisms (Singh, 1987).

Taiwo and Oso (2004) reported mesophilic bacterial cultures such as *Bacillus sp,* coliforms, *Pseudomonas, Streptococcus, Proteus* and *Serratia* at the early stages of composting. However, *Bacillus spp*, is isolated at the mesophilic stage. At the peak of composting, the number of actinomycetes and bacteria declined and thermophilic fungi recolonizes. Mishra *et al.,* (1982) reported *Chaetomium globosum, Fusarium solani, Paecilomyces varioti*, and *Penicillium chrysogemem* were common in compost piles. The selection of suitable microbes depends on the type of composting process i.e. aerobic or anaerobic, type of raw materials, etc. the efficient cellulolytic

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cultures, such as species of *Aspergillus, Trichoderma*, *Penicillium* and *trichurus* accelerate composting for efficient recycling of pressmud with high C:N ratio and reduce the composting period about 1 month. Natural cellulose substrates (Primarily plant cell materials) are composed of heterogeneous intertwined polysaccharide chains with varying degrees of crystallinity, hemicelluloses, xylene and pectins, embedded in lignin, microorganisms produce multiple enzymes to degrade plant cell materials known as enzyme systems (Prabhu and Maheswari, 1999). For microorganisms to hydrolyze and metabolize insoluble cellulose, hemicellulose, xylan, pectin and lignin, extracellular endo and exo enzymes must be produced that are either free or cell associated (Lynd *et al*., 2002). This current study involves use of microbial consortia on composting pressmud waste from sugar industry and subsequently made suitable for soil application more focus on the study is on extracellular enzymatic systems on composting pressmud waste from sugar industry.

MATERIALS AND METHODS

Windrow composting technology was carried out at the composting yard of m/s thiru Arooran sugars Ltd., Tamilnadu, India. Heaps of pressmud were formed in triangular shape with 1.5 m height and 2.1 m width at the bottom and 25m length. Each row is called windrow. The Windrow were sprayed with spent wash to a moisture level of 60 to 70 percent. The ratio of pressmud and spent wash is to be 1:2. Selected microbial cultures namely, *Bacillus spheroticus, Aspergillus fumigatus, Trichoderma reesi* employed for composting of press mud is mixed with distillery spentwash. The treatment details and as follows, T_1 - *Bacillus spheroticus*, T_2 - *Aspergillus fumigatus*, T_3 - *Trichoderma reesi alone*, T_4 - *Bacillus spheroticus* + *Aspergillus fumigatus*, T_5 - *Bacillus spheroticus* + *Trichoderma reesi*, T_6 - *Aspergillus fumigatus* +*Trichoderma reesi,* T_7 - *Bacillus spheroticus, Aspergillus fumigatus, Trichoderma reesi* T_8 – control, the designs followed were randomized block designs with appropriate replications, respective treatment inoculants were added at the rate of 2 liters per tonne of pressmud Specially developed microbial inoculants and consortia were added at the rate of 2 lit/tonne of pressmud. This is diluted to 10 liters of water for uniform spraying on the pressmud. The specially designed machine called “Aero tiller” is to be used for turning the pressmud and also for uniform spreads of microbial inoculum to the entire length of windrows. Spent wash spraying activities continued till the end of 6 weeks. The aero tiller machine was moved over the windrows periodically, every windrow was turned at least four times in a week followed by trimming of windrows and spent wash application up to 45 days.

Then allowed for curing by maintaining moisture level of 50 – 60%. The treatment compost samples were assayed for Endo and Exo pectinase, Endo and Exo Cellulases and xylanases starting from 0^{th} day of inoculation up to 90 days with fifteen days intervals in between one gram of compost sample was taken in each 50ml Erlenmeyer flasks and to this respective buffers and substrates were added for further enzyme assays. Endo and Exo cellulases activity were etc. mated by the method of Schinner and Von mersei (1990) using 0.5% carboxy methyl cellulose as substrate. Endo pectinases were assayed by the method discovered by Hancock and Semb, (1963) using 0.5% pectin as substrate and Exopectinases were assayed by the method of wang *et al* (1997) using 0.5% polygalacturon as substrate, xylanase activities were estimated by the method of sachinner and Von Mersei (1990) using 1% xylan as substrate. The changes in pH, Ec (dsm^{-1}) and C: N ratio at different periods of composting of pressmud with distillery spentwash were recorded from 0 days onwards upto 90 days with fortnight intervals (Jackson, 1967, Jackson, 1973; Lossin, 1970). All determination were carried out in triplicates and CD values at p = 0.05 were used to determine the significant difference between treatment means.

RESULTS AND DISCUSSION

Microbial enzymes play a vital role in composting process. The composting technologies require an efficient microbes or consortia to reduce the composting periods in order to minimize the lime cost and management of wastes. The endo 1, 4 gluconases activity was higher between 30 to 45 days of composting the *Trichoderma reesci* (T_3) was formed to record 61.48% reduction on viscosity on 30^{th} day of composting. The triple inoculant consortium treatment (T_7) resulted comparatively higher endo cleaving ability starting from 30^{th} day upto 45 days of composting (Table–1). All the treatments showed reduction in endo glucanases activity after 45 days of composting. Goyal *et.al.* (2005) reported that cellulose activity was maximum on 30^{th} day in all treatments and declined there after upto 90 days. The low cellulose activity after 45 days of composting may be attributed to the relative proportion of lignin in the composting or exhaustion of substrate. The exo -44 glucanases activity starting from 30^{th} day onwards, sustained their enzyme production upto 60^{th} day and sudden drop there after (Table - 2). The consortium treatment (T_7) performed better than the other treatments. The maximum enzyme production was recorded on 30^{th} day (1.72 mg^{-1}) of protein by the treatment T_7. The dual inoculum treatment T_6 showed maximum endo poly galacturanase on 45^{th} day (53.24% reduction in viscosity) followed by T_5 and T_4 (Table – 3). The consortium triple inoculum treatment showed maximum endo polygalacturonase activity on 45^{th} day was 55.48% reduction in viscosity. The endo polygalacturonase activity observed a peak on 45^{th} in all treatments and there after decreases. Ryckelbolr *et.al.* 2003 reported pectinolytic activity reacts its maximum between 30 and 45 days in all treatments. The pressmud is a complex molecule containing cellulose hemicellulose, pectin and lignin.

The ability of selected population to degrade complex molecules were affected in the presence of lignin after 45 days after composting. The exo polygalacturonase activity recorded during composting periods (Table-4). The individual inoculants treatments revealed that T_3 recorded maximum exo PG on 45^{th} day (1.12 mg-1 of protein) followed by T_2. The triple microbial consortium showed maximum exo PG activity from 30^{th} day onwards and the activities sustained upto 75 days of composting. Gupta *et.al.* (1987) reported the polygalacturonase activity was induced at 1% pectin in concentration in *Alternaria alternata*. The pectin serves as both inducer reducer depending on concentrations.

Table 1 Production of Endo – 1, 4 glucanase activity during composting periods

Sl. No	Treatments	Endo β-1,4 glucanase (% reduction in viscosity) Composting period (days)						
		0	15	30	45	60	75	90
1.	T1 - *Bacillus sphaeroticus*	8.36	12.23	59.83	59.43	45.33	33.28	30.12
2.	T2 - *Aspergillus fumigatus*	8.42	15.16	60.00	58.12	48.24	40.17	36.29
3.	T3 - *Trichoderma reesei*	8.38	18.46	61.48	60.3	50.21	52.38	45.69
4.	T4 - *B.Sphaeroticus + A. fumigatus*	8.63	16.73	69.13	57.38	48.17	40.13	38.16
5.	T5 - *B.Sphaeroticus + T.reesei*	8.47	15.83	65.39	64.23	50.12	49.82	42.16
6.	T6 – *A. fumigatus + T.reesei*	8.49	13.24	63.21	59.27	50.13	45.38	37.33
7.	T7 - *B.Sphaeroticus + A. fumigatus + T.reesei*	8.51	11.33	71.28	70.13	51.23	49.23	41.06
8.	T8 Control	8.9	13.65	33.86	24.17	18.63	16.17	15.78
	SE	0.88	0.76	0.92	1.18	1.02	1.34	1.42
	CD (0.05)	N.S	1.53	1.90	2.20	2.05	2.72	3.98

Table 2 Production of Exo 1,4 glucanase activity during composting periods

Sl. No	Treatments	Exo 1,4 glucanase (U mg^{-1} of protein)						
		Composting period (days)						
		0	15	30	45	60	75	90
1.	T1 - *Bacillus sphaeroticus*	0.09	0.33	1.28	1.33	0.98	0.86	0.72
2.	T2 - *Aspergillus fumigatus*	0.12	0.37	1.36	1.28	1.04	0.93	0.87
3.	T3 - *Trichoderma reesei*	0.1	0.32	1.02	1.3	1.01	1.47	1.12
4.	T4 - *B.Sphaeroticus + A. fumigatus*	0.08	0.35	1.33	1.3	0.92	0.84	0.71
5.	T5 - *B.Sphaeroticus + T.reesei*	0.09	0.31	1.42	1.4	1.01	0.93	0.88
6.	T6 – *A. fumigatus + T.reesei*	0.09	0.34	1.52	1.39	1.1	0.96	0.87
7.	T7 - *B.Sphaeroticus + A. fumigatus + T.reesei*	0.11	0.39	1.72	1.54	1.04	0.97	0.84
8.	T8 Control	0.09	0.31	0.68	0.43	0.32	0.28	0.26
	SE	0.01	0.02	0.13	0.15	0.13	0.15	0.11
	CD (0.05)	N.S	0.07	0.27	0.33	0.28	0.31	0.24

Table 3 Production of Endopolygalacturonase activity during composting periods

Sl. No	Treatments	Endo polygalacturonase (% reduction in viscosity)						
		Composting period (days)						
		0	15	30	45	60	75	90
1.	T1 - *Bacillus sphaeroticus*	5.28	11.23	49.27	43.27	34.27	31.17	26.14
2.	T2 - *Aspergillus fumigatus*	5.34	11.18	48.18	41.12	32.16	30.13	29.10
3.	T3 - *Trichoderma reesei*	5.08	11.1	40.27	44.25	32.8	31.18	29.13
4.	T4 - *B.Sphaeroticus + A. fumigatus*	5.17	11.24	48.13	44.18	33.8	30.15	28.16
5.	T5 - *B.Sphaeroticus + T.reesei*	5.29	11.34	50.8	50.01	40.23	39.17	37.25
6.	T6 – *A. fumigatus + T.reesei*	5.31	11.68	50.13	53.24	39.43	31.17	30.09
7.	T7 - *B.Sphaeroticus + A. fumigatus + T.reesei*	5.38	11.29	51.23	55.48	52.13	47.23	45.13
8.	T8 Control	5.47	10.96	24.18	20.14	17.67	16.28	15.23
	SE	0.54	1.15	0.55	0.91	0.75	1.01	1.10
	CD (0.05)	N.S	0.38	1.07	1.99	1.50	2.03	2.20

Table 4 Production of Exo polygalacturonase activity during composting periods

Sl. No	Treatments	Exo polygalacturonase (U/mg of protein)						
		Composting period (days)						
		0	15	30	45	60	75	90
1.	T1 - *Bacillus sphaeroticus*	0.06	0.12	0.82	0.63	0.23	0.19	0.18
2.	T2 - *Aspergillus fumigatus*	0.07	0.09	0.96	1.02	0.43	0.33	0.28
3.	T3 - *Trichoderma reesei*	0.07	0.13	0.38	1.12	0.46	0.38	0.29
4.	T4 - *B.Sphaeroticus + A. fumigatus*	0.09	0.16	0.73	0.94	0.51	0.42	0.31
5.	T5 - *B.Sphaeroticus + T.reesei*	0.1	0.12	0.97	1.06	0.63	0.46	0.38
6.	T6 – *A. fumigatus + T.reesei*	0.07	0.1	0.95	1.00	0.72	0.60	0.43
7.	T7 - *B.Sphaeroticus + A. fumigatus + T.reesei*	0.08	0.13	0.92	1.13	1.00	0.8	0.52
8.	T8 Control	0.08	0.12	0.14	0.09	0.03	0.02	0.02
	SE	0.01	0.02	0.04	0.13	0.03	0.04	0.02
	CD (0.05)	N.S	0.04	0.08	0.07	0.06	0.08	0.04

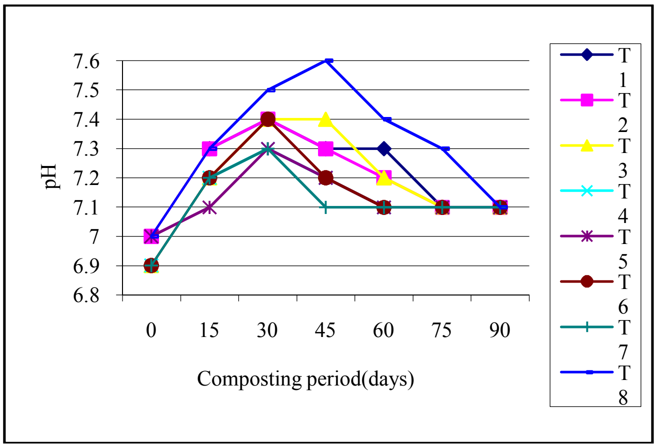
The significant reduction in exo PG activity after 45th day may be attributed to the available pectin in the pressmud sample that was being composted. The peak xylanase activity in all treatments was found to be on 30th day (Table – 5). There after a sharp decline was noticed.

Among the treatments, T_7 recorded maximum xylanase activity on 30th day of composting 35.25 IU ml^{-1}. Goyal *et.al.* (2005) reported celluloses and xylanases showed proportionate reduction in cellulose and hemicellulose from first to 60th day of composting of water hyacinth, poultry waste and pig slurry.

Table 5 Production of Xylanase activity during composting periods

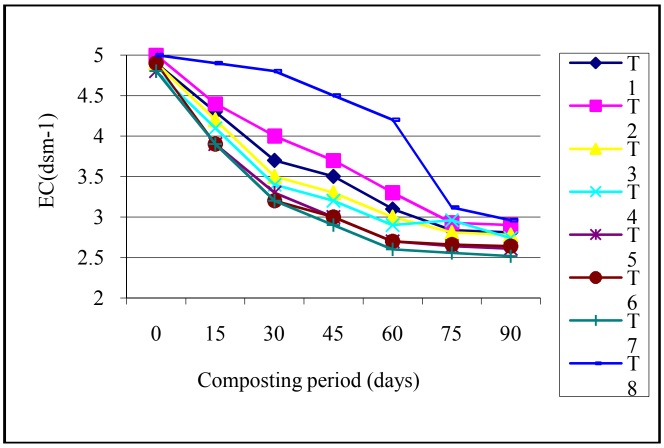
Sl. No	Treatments	Xylanase (IU/ml) Composting period (days)						
		0	15	30	45	60	75	90
1.	T1 - *Bacillus sphaeroticus*	0.09	1.24	19.12	9.68	4.28	3.96	3.01
2.	T2 - *Aspergillus fumigatus*	0.08	1.27	21.68	9.72	3.29	3.09	2.96
3.	T3 - *Trichoderma reesei*	0.08	1.31	19.36	9.43	4.23	4.17	3.94
4.	T4 - *B.Sphaeroticus + A. fumigatus*	0.06	1.22	26.28	10.12	6.23	5.13	4.92
5.	T5 - *B.Sphaeroticus + T.reesei*	0.08	1.23	28.16	8.37	5.13	4.78	3.96
6.	T6 – *A. fumigatus + T.reesei*	0.07	1.29	29.98	6.78	2.43	2.23	2.01
7.	T7 - *B.Sphaeroticus + A. fumigatus + T.reesei*	0.06	1.36	35.23	12.13	6.93	6.28	5.93
8.	T8 Control	0.08	1.33	11.27	6.12	2.13	2.01	2.01
	SE	0.01	0.05	1.13	0.27	0.11	0.09	0.10
	CD (0.05)	N.S	0.11	2.27	0.47	0.22	0.20	0.20

This may be understood that physio chemical properties of the substrate and the availability of protein ion water hyacinth may delayed the xylolytic activity upto 60 days. However pressmud does not contain higher proteinaceus substrates and not comparable to the water hyacinth, poultry waste and pig slurry. In general all treatment recorded peak of xylase activity on 30th day of composting and considerable reduced after 75th day. Comparative studies on changes in p^{H} at different periods of composting of pressmud revealed that, P^{H} starts to raise from 15th day onwards (Fig.1). The consortium treatment (T_7) attains stability in P^{H} (7.1) on 45th day itself whereas the dual inoculum treatments, T_4, T_5 and T_6 required another 15 days (60 days).



Figures 1 Changes in pH at different periods of composting of pressmud and distillery Spent wash

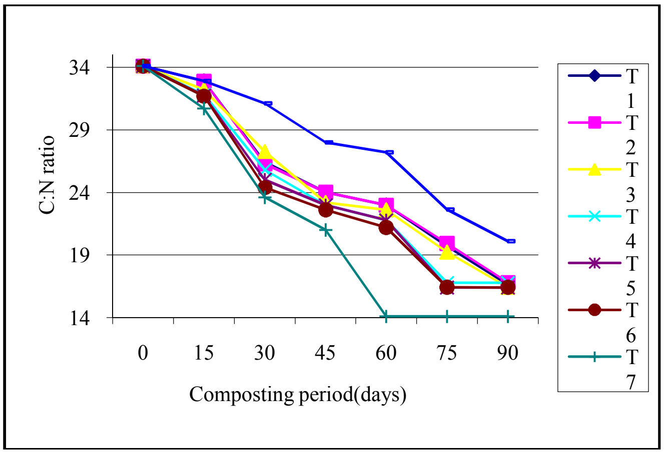
The pressmud is moistened with spentwash and their initial pH of pressmud 6.3, treated spent wash pH 7.6. Addition of spentwash, on alternate days in order to maintain moisture 60 – 70% enable the pH content of the compost between 7.0 to 7.4 further, microbial activity and their extra cellular enzyme production helps to attain stability in pH on 45th day. Datta and Gupta (1983) reported EC of soil was increased by the application of spentwash. When spent wash was mixed with pressmud the EC content of pressmud brought 4.8 to 5.0 dsm^{-1}. In consortium treated (T_7) pressmud compost the lowest EC 2.9 dsm^{-1} achieved on 45th day while the dual inoculant treatments requires 60 days to active Ec 2.9 (dsm^{-1}) and in the single inoculant treatment compost the EC of 2.9 (dsm^{-1}) was achieved only on 75th day (Fig. 2).



Figures 2 Changes in electrical conductivity (dsm-1) at different periods of composting of pressmud and distillery Spent wash

Taiwo and OSO (2004) reported the change in EC by various treatment attributed to the microbial action and extra cellular enzyme complexes on composting materials. The changes in C: N ratio at different periods of composting directly correlated to the maturity of the compost.

Houseeini *et.al.* (2002) reported that action of mesophilic and thermophilic organisms in a succession could reduce C: N ratio of substrate to 25.30:1 within 60 days after composting. The T_7 treatments attains the C: N ratio of 21:1 within 45 days (Fig.3). The dual inoculum treatments T_4, T_5 and T_6 required 60 days of single inoculant treatments T_1, T_2 and T_3 required 75 days.



Figures 3 Changes in C:N ratio at different periods of composting of pressmud and distillery spent wash

The microbial activities are directly related to the availability of energy sources and inorganic nutrients required for their growth. Further extra cellular enzyme complexes exhibited by native and introduced population drastically influenced by the physico chemical properties of the substrate. The microbial succession over the periods of composting directly related to the early maturity of any compost. In general the study recalled that the single inoculant treatment fails to compost the pressmud not earlier than 75 days. Whereas dual inoculants could achieve maturity of the compost with in 60 days. However the selected triple inoculant consortia could enforce an early maturity within 45 days. The selected triple microbial consortia (T_7) used for composting of pressmud contained a mesophilic of thermophilic. The presence of these organisms throughout the periods of composting and their ability to secrete extracellular enzyme complexes could pave the way for an early maturity. Further, a detailed account on inhibitors during the degradation of the presence of metabolic pressmud will tailor the efficiency of extracellular enzyme complexes, in order to enhance (or) hasten the process of composting.

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