Research Article

To Study the Microbial Diversity and Its Biological Activity During Composting and Vermicomposting of Urban Solid Waste

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ABSTRACT

Urban solid wastes are the potential degradable, organic matter available as a source of plant nutrients. Decomposition of this waste material under regular composting and vermicomposting was carried out to understand the process management, microbial density occurring at different stages of decomposition and their enzymatic effect on the process and to assess the manorial value of the developed compost. Experiments were conducted to know the biochemical characteristics and total population of beneficial microorganisms during composting and vermicomposting of urban solid waste. And inoculation effect of isolated beneficial microorganisms on the growth of French bean.

Urban solid wastes were initially subjected to decomposition for 30 days. A batch of equal quantity was allowed for compositing and to the other batch earthworms were released for vermicomposting. The enumeration of beneficial bacteria and fungi in the compost and vermicompost samples were also carried out. Bacterial population was maximum at initial 15days of decomposition and declined at 90days. Similar trends were also observed in vermicomposting process. Fungi population also gave similar results expect in vermicomposting samples. Total reduction in total carbon and nitrogen content were vermicompositing showing least (10.22) C/N ratio composed to compost (11.77) on 90th days.

The nitrogen, phosphorus and potassium gradually increased with period of decomposition but did not show any difference in content under both system of composting. The cellulose degraders number more in vermicompost as compared to compost on 45th day. And the trend continued till 90th days. Effects of compost material and beneficial microorganisms on plant growth were studied. A combination of regular compost with nitrogen fixing actinomyces (NFA) showed maximum growth was for bean in height and maximum increase in leaf number in treatment inoculated with PSB9. A nitrogen fixing bacteria (NFB11) with vermicompost has resulted in a significant increase in plant growth with highest biomass as compared to the use of other beneficial microorganisms over control. The results indicated that the compost and vermicompost with beneficial microorganisms can be effectively used for the better plant growth and yield.

Keywords: Actinomycetes, Biomass, Microorganisms, Urban solid waste.

INTRODUCTION

The soil fauna including earthworms, termites, soil micro arthropods, millipedes, nematodes, mollusks etc., played an important role in increasing the rate fog microbial decomposition and mineralization as the food passed through their guts and made the organic matter in to small fragments[1]. Earthworm species such as *Apporrectodea coliginona*, *Apporrectodea lona, Lumbricus rubellus, lumbricus.teerestris* earthworm species showed a strong preference for pure mineral soil over pure organic matter[2]. Eleven common species of earthworm, which could be utilized for improving soil fertility and garbage decomposition. Bacterial numbers accompanied by a rise in carbon dioxide production. It was therefore concluded that the rate of decomposition of organic matter in soil were a function of microbial activity[3]. The fungi was more efficient in their use of organic substrates in frest soil and he observed that fungi have competitive advantage over bacteris[4]. The microbial respiration and enzyme activity with increasing application rates of sewage sludge, compost and sewage sludge mixed with coal sludge to soils[5]. The disposal of solid urban wastes in asian countries, stated that for compositing under aerobic conditions the optimum intial C/N ratio between 26 and 31, with was greater agreement in this ares of work, that if the initial C/N ratio was greater than 30, there will not be appreciable loss of nitrogen as ammonia during composting[6]. High temperature condition was necessary for good compositing. And also reported that organic matter with a wide range of PH (0.3

to 11.0) can be compositing end product[7]. During decomposition promoted the conversation of carbohydrates in to carbondioxide and water. Incomplete aeration may result in accumulation of acetic acid and thus affect the plant growth when the compost is incorporated into the soil. Proteins and aminoacids are converted to ammonia and microbial protein[8]. The organic acids produces by phosphate solubilizing microorganism were oxalic acid, succinic acids, uric acids and 2-Keto gluconic acid[9]. Vermicastings are rich sources of macro and micro nutrients vitamins, enzymes, antibiotics, growth hormones and immobilized micro flora[10]. The addition of compost increased the soil PH , availability of nitrogen, phosphorus, potassium and calcium compared to mineral fertilizers[11]. Free living nitrogen fixing bacteria isolated from ten graminaceous species of which *Clostridium*, *Desulfovibrio*, *Klebsiella* and *Azospirillum* were more predominant[12]. The total loss of P and N forms were decreased in surface runoff from pasture when the production of earthworm casts was observed compared to the lake of earthworm casts[13]. The urease activity of the soils were variable and also inhibition activity was effectively achieved using Ag+, while Cu+ were only effective in two soils[14]. The rapid decomposition of sewage sludge in the early days of application but actionmyces and fungi contributes to the gradual decomposition after the initial rapid decomposition[15]. The yield of the long duration crops like sugarcane and banana were increased due to application to compost and farm yard manure[16]. The effect of vermicompost on cardamom nursery seedlings. Results indicated that vermicompost is superior to normal compost in increasing to application of FYM and urea or urea and crop residues[17]. The performance of French bean Cv. Arka Komal using FYM and vermicompost as organic nutrient sources and ammonia sulphate as inorganic nutrients source. The study indicated that nitrogen uptake were significantly high from inorganic source during pre flowering stage, whereas phosphorous and potassium uptake from three sources were on par. Nodulation were highest in FYM treated soils followed by environment[18].

MATERIALS AND METHODS

Sample collection

The urban solid wastes generated in the Chennai city residential areas were collected and converted to compost at the plant site at porur.

Sperber's Medium and Norrin's medium

100 ml portions of the medium were dispersed to 250ml flask and 1.8g agar added to each flask. K_2HPO_4 and $CaCl_2$ solution added aseptically and the media is sterilized when the media were 60^o c. 5ml of $CaCl_2$ and 2ml of K_2HPO_4 solution were added to obtain white insoluble precipitate of hydroxyl apatite then poured in to plates.

Composting of urban domestic solid wastes

The organic material were collected every day from the domestic areas were added to the composting tank layer by layer for 7 days. Aeration was provided by turnings at 15 days interval for 30 days and initially composting was carried out for 30 days.

Vermicomposting

Initial decomposition of the wastes, they are divided in to two parts. One part were used for vermiculture and mixed species of earthworms *Endrillus eugeneae, Peryonyx excavates* and *Eusenia foetida.* Were added at the rate of 500g for 500 Kg of wastes and was further decomposed for a period of 60 days. Other part of the decomposed materials was used in regular composting process without any earthworms for 60 days. In this process, aeration was provided by turnings.

Determine of microbial diversity in compost

Microbial diversity during decomposition process were studied with regard to the occurrence and distribution of bacteria, fungi, actinomyces, nitrogen fixers, phosphate solubilizers, cellulose degrades and protein degrades.

Isolation and enumeration micro organisms

Nitrogen fixers and phosphate solubilizers were isolated from the compositing materials at 0, 15, 30, 45, 60 and 90 days respectively. Norrin's medium and sperber's medium were used for enumeration and growth of N2 –fixers and P-solubilizers respectively. After two days fo plating, P-solubilized clear zones were observed on Sperber's medium and N2 colonies were observed on Norrin's medium. The pure culture were maintained in test tubes slants and preserved to carry out for further experiments.

Enzyme activity

Urease activity

5g each of freshly collected samples were placed in a volumetric flask, 0.2 ml of toluene and 9 ml of tris buffer (PH 9) and 1 ml of 3% urea solution were added and incubated for 2 hours at 37^{o}C and to filtered through Whatmann No.42 filter paper. From this solution 1% Sodium tartarate and 1% gum acacia solution and 5ml nessler's reagents were added. The yellow colour developed after 30 minutes were measured at 410nm using Spectrophotometer.

Cellulose activity

10g of compost sample was placed in Erlenmayer flask. 15ml of acetate buffer followed and 15ml of Carboxymethyl cellulose solution were added and incubated for 24 hr. After add 5ml of ferric ammonium sulphate , sodium dodecyl sulphate and concentrated sulphuric acid were mixed well and kept for 60 minutes for color change development and then blue colour were observed exactly after 30 minutes at 710nm, by using Spectrophotometer.

Total nitrogen

2g of digestion mixture containing potassium sulphate, cpper sulphate and selenium in the ratio 100:20:1.

Total potassium

Potassium by using flame photometer.

Total phosphorous

500mg dried and powdered samples of compost and vermicompost were digested with 10ml of triacid mixture on a hot plate until dense brown fumes evolved.

PH

The pH were determined by potentiometry using combined glass electrode.

Compost and Vermicompost enrichment

The final compost and vermicompost were inoculated with 10 days old culture and inoculated for 7 days. The cultures introduced were P-solubilized and N2 – fixers.

1. Bacterial isolates-PSB9
2. Fungal isolates –PSF13
3. Bacterial isolates- NFB11
4. Actinomyces isolates- NFA

Effects of enriched compost and vermicompost on French bean growth

Soil Alfisols of GKVK was used for growimg of French beans and the soil. C/N ratio

The experiment were carried out in green house on French beans variety.

T1= Vermicompost + Inoculated with microbial culture PSF 13
T2= Vermicompost + Inoculated with microbial culture PSB 9
T3= Vermicompost + Inoculated with microbial culture NFB 11
T4= Vermicompost + Inoculated with microbial culture NFA

Regular compost

T1= Regular compost+ Inoculated with microbial culture PSF 13
T1= Regular compost+ Inoculated with microbial culture PSB 9
T1= Regular compost + Inoculated with microbial culture NFB 11
T1= Regular compost + Inoculated with microbial culture NFA.

Biomass of plant

Harvested shoots and roots to constant weight were dried for 5 to 7 days.

Plant nutrient uptake

The shoots samples were analysed for total NPK

Statistical analysis

The various observation and data recorded in the experiment were analysed statistically using standard procedure.

RESULTS AND DISCUSSION

Microbial changes during the decomposition of urban solid wastes comprising of domestic wastes and other miscellaneous degradable wastes. The composting process were compared and two different methods, namely regular composting and vermicomposting. The changes in pH total carbon, total nitrogen and C/N ratio during decomposition is presented in Table a. Total carbon showed that it gradually during the initial decomposition period upto 30 days from 46.47% to 33.84%. On the vermicomposting the rate of decomposition were faster with reduction in total carbon from 37.71% at 45 days to a lowest of 18.0% at end of the process whereas during the regular compositing, the rate of decomposition were low upto 60 days and percent carbon reduced to 19.91. Initial N content of 1.04% to 1.34% on 30th day in both composting.

Table a: Change in pH, carbon and C/N ratio during decomposition

NO OF DAYS	PH		TOTAL C%		TOTAL N%		C/N	
	RC	VC	RC	VC	RC	VC	RC	VC
0	5.3	5.2	45.47	46.47	1	1.04	44.87	44.77
15	6.8	6.8	38.6	38.6	1.22	1.22	31.63	31.63
30	6.9	6.9	33.84	33.8	1.34	1.34	25.25	25.25
45	7.2	7.4	32.25	31.71	1.48	1.56	21.79	20.32
60	7.4	7.8	32.2	27.9	1.6	1.64	20.15	17.17
90	7.8	8.1	19.9	18	1.6	1.76	11.71	10.22

And vermicomposting which further increased to 1.70 during regular compositing and to 1.76% under vermicomposting, which also showed a higher N content at different stages of vermicomposting. Initial stages wherein the C/N ratio significantly reduced from 44.87 to 22.25 in 30 days. A lowest ration of 11.71 at 90 days in regular composting to vermicomposting having a C/N ratio of 10.2%(Table a)
Changes in total nitrogen, total phosphorous and potassium content during different stages of decomposition in Table b).

Table b: Changes in total nitrogen, total phosphorous and potassium during decomposition

DAYS	TOTAL N mg/g		TOTAL P mg/g		K mg/g	
	VERMICOMPOST	COMPOST	VERMICOMPOST	COMPOST	VERMICOMPOST	COMPOST
0	10.4	10.4	9.1	9.1	5.1	5.1
15	12.2	12.2	17.1	17.1	6.1	6.1
30	13.4	13.4	23.8	23.8	7.95	7.95
45	15.6	14.8	27	27.5	12.3	14.42
60	16.4	16	27.9	28.6	11.4	12
90	17.6	17	28.1	28.5	12.3	13

Population density of bacteria, fungi and actinomycetes at different stages of regular and vermicompositing is presented in Table 3. Initial bacterial decomposition is 99.14 CFUx10 and reduced to 33.19x10 on 30th day. Vermicomposting process increased the bacterial population from 30th day onwards to a maximum density of 97.74CHUx10g on 60th day there after declined to 89.6 CFUx10g by 90 days. Fungal population recorded an increase with decomposition and reached population of 59.7x10g on 30th day compositing in both the compositing process. Actinomyces population recorded an increase only after 15th days of initial composting, with an initial population density of 11.24 CFUx10g at 0day compositing in both the process (Table c)

Table c: Population of different groups of microorganisms during the process of decomposition

TREATMENTS	MICROBIAL POPULATION					
DAYS	BACTERIAL CFUx10^5 /g		FUNGAL CFUx10^5 /g		ACTINOMYCETES CFUx10^5 /g	
	VERMICOMPOST	COMPOST	VERMICOMPOST	COMPOST	VERMICOMPOST	COMPOST
0	84.8	84.8	22.1	22.1	11.24	11.24
15	99.1	99.1	29.6	29.5	11.25	11.25
30	33.19	33.19	59.7	59.6	44.6	44.5
45	46.8	54.97	56.6	56.7	55.19	40.72
60	97.74	92.3	42.5	41.25	82.51	66.57
90	89.6	94.7	37	30.1	59.97	44.81

The population increased from 1.36x10 to 1.41x10 CFU/g in both regular compositing and vermicompositing. The vermicompositing process increased the cellulose degrades to a maximum of

1.66x10 on the 45th day which further reduced on 60th and 90th day. The population of protein degrades the initial stage 193x10/g reduced to 174.4x10 CFU by 30 days in vermicompost and were maximum on the 90th with a population of 28.3x10 CFU compared to vermicompost (36.5x10 CFU/g). (Table d)

Table d: Influence of compositing processes on population density of phosphate solubilizers and nitrogen fixers

DAYS	CELLULOSE CFUx10^5 /g		PROTEIN CFUx10^5 /g	
	VERMICOMPOST	COMPOST	VERMICOMPOST	COMPOST
0	1.36	1.36	193	193.06
15	1.49	1.49	168.7	168.7
30	1.41	1.41	174.4	174.42
45	1.66	1.37	148.6	124.4
60	1.49	1.29	96.5	63.5
90	1.28	0.99	36.5	28.13

Vermicompositing process the phosphate solubilising microorganisms significantly increased from a population level of 172.29x10CFU/g at 45 days to 352.17CFU/g on 90th days. Similar trend were recorded for regular compost with a lower level of 160x10CFU at 45 days to 311.9x10 on 60 days but reduced to 235.5x10CFU on 90th days (Table e)

Table e: population density of phosphate solubilising and nitrogen fixers

DAYS	P-solubilizer CFUx10^5		N-fixers CFUx10^5	
	VERMICOMPOST	COMPOST	VERMICOMPOST	COMPOST
0	228.05	228.06	19.21	19.21
15	79.04	79.04	6.35	6.36
30	92.29	92.28	2.6	2.6
45	172.3	160.3	14.75	14.26
60	341.2	311.9	29.96	29.23
90	352.2	235.5	17.8	22.8

Cellulase activity were gradually increased from an initial activity of 1.252ug reducing sugars/g to 5.9 ug/g in15days and 0.29 ug/g on 30th days.To increase in activity were higher during compositing process by 60th day to 1.09 mg reducing sugar/g to 1.11ug on 90th days and the vermicompositing was of lower levels. The urease activity (113.8ug) by 15th days to 1.6ug which slightly increased next 15th days and was very least on 90th days having activity of 0.4ug (Table f).

Table f: Influence of compositing processes on urease and cellulose activities

DAYS	CELLULASE ACTIVITY		UREASE ACTIVITY	
	VERMICOMPOST	COMPOST	VERMICOMPOST	COMPOST
0	1.25	1.25	113.8	113.8
15	5.99	5.99	32.4	32.4
30	0.3	0.29	44.6	44.6
45	0.89	0.92	1.6	7.8
60	1.05	1.08	2.6	3.8
90	1.04	1.12	0.4	1.03

Efficiency of nitrogen fixation capacity of the predominant bacterial isolates is presented in the Table 6. Crude protein content of 8.22% followed by NFB11 with 12.9mg N and Actinomyces, isolate NFA showed Nitrogen fixation of 11.55mg N. Nitrogen uptake was maximum in treatments amended with microbial cultures PSF9 and PSF13 (10.57 mg and 8.89mg/pl respectively). PSB9 microbial culture with a maximum K-uptake of 54.82 and 47.7mg/pl and bacterial isolates and fungal isolate PSF13, (NFA) axtinomycete inoculation did not show any increase in K uptake. Maximum uptake of Nitrogen were in treatment (T5) amended with NFA followed by NFB11 with 63.80 and (40.99mg/plant). Treatments with NFB11 and NFA showed highest values of K update (75.22 and 63.58mg/pl respectively) (Table g and Table h).

Table g: Effect of regular compost on nutriment uptake by French bean (*Phaseolus vulgaricus*)

TREATMENT	N-UPTAKE mg/pl	P-UPTAKE mg/pl	K-UPTAKE mg/pl
T1:RC+PSF13	50.83	8.98	39.7
T2:RC+PSB9	50.9	6.27	47.7
T3:RC+NFB11	50.24	2.71	41.29
T4:RC+NFA	50.2	7.8	49.5
T5+CONTROL	15.28	1.7	19.2

Table h: Effect of vermicompost on nutriment uptake by French bean (*Phaseolus vulgaricus*)

TREATMENT	N-UPTAKE mg/pl	P-UPTAKE mg/pl	K-UPTAKE mg/pl
T1:VC+PSF13	44.9	5.32	27.7
T2:VC+PSB9	35.4	3.53	35.0
T3:VC+NFB11	63.2	5.65	75.22
T4:VC+NFA	63.8	7.37	63.6
T5+CONTROL	15.28	1.7	19.2

Table i) showed that inoculation of actionmycetes having nitrogen fixing property (NFA) influenced plant growth. However the maximum of leaves were due to NFB 11, a Nitrogen fixing bacterial isolate (14 leaf /plant). Dry matter production were maximumdue to PSB9 a phosphate solubilizing bacterial isolate (2.12g/plant) and fungi (3.87g/pl).

The vermicomposting on plant growth, the number of leaves produced were maximum for nitrogen fixing bacterial isolates NFB11 with leaves followed by Phosphate solubilizing fungal isolates PSF13 (12.8). The dry biomass production were found to be maximum due to nitrogen fixing bacterial NFB11 and Actinomycetes NFA with 2.20 and 2.18g/pl respectively.

Table i: Biomass production of French bean- Evaluvation of regular compost and vermicompost

TREATMENTS	ROOT DRY WEIGHT	SHOOT DRY WEIGHT	TOTAL BIOMASS
T1:RC+PSF13	1.69	1.79	3.48
T2:RC+PSB9	1.75	2.12	3.87
T3:RC+NFB11	1.34	1.66	3
T4:RC+NFA	1.54	1.7	3.23
T5+CONTROL	0.64	0.9	1.62

Plate 1A
Inoculation of phosphobacterial isolate in compost on growth of *Phaseolus vulgaricus*
VC PSB9= Vermicompost+ PSB, VC Control =Uniinoculated vermicomposting
Plate 1B-Inoculation response of actiomycetes in compost on *Phaseolus vulgaricus*
Plate 2-Response of regular compost on shoot and root growth of *Phaseolus vulgaricus*
Shoot and Root growth
NPK, PSB9, NFB11, PSF13 , Control.
Plate 3- Inoculation response of regular compost- RC PSF13 is bacterial inoculation and RC PSB9 : Fungal inoculation.
Plate 3A- Comparision of vermicompost with recommended NPK, No vermicompost, No fertilizers.
Plate4- Microbial inoculation response of vermicomposton French bean. VC PSB9 Phosphate solubilizing bacteria and VC PSB13 Nitrogen fixing actinomycetes. Plate 5- Actinomycetes isolate (NFA) from vermicompost.

CONCLUSION

Composting is a familiar process from the product oriented perspective of producing an organic manure for application to soil. The treatment orientation emphasized in this study is the process management influences by different microbial population. Managing their biochemical activities to maximize the rate of decomposition would be a cost effective method of waste treatment. This would help in routine handling of urban solid wastes. Hence, by proper management process of a waste that is not acceptable for disposal at a land fill to a process residue that is acceptance.

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