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Production of microbial rich substrate from bio-solid wastes

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Abstract

This study evaluates the potential of municipal solid waste (MSW) as a substrate for vermicomposting when amended with organic supplements such as cow dung (CD) and mango litter (ML). Ten vermibeds were prepared using different substrate ratios (MSW, CD, and ML) and inoculated with the earthworm Eisenia fetida. *After 60 days of treatment, the resulting vermicompost showed a marked reduction in organic carbon (OC) content. The maximum loss in OC over the initial levels was 57.31%, accompanied by significant increases in total nitrogen (N) the highest rise in total N content recorded was 26.31%, available phosphorus (P) 72.72% and potassium (K) compared to initial concentrations 60.75%. Microbial populations also exhibited a notable increase across all treatments. The highest bacterial population in the vermicompost obtained was 610.12±1.83 CFU×10^6 while the highest number of fungal colonies in the end product obtained were 269.14±0.83 CFU×10^4. The results demonstrate that the addition of CD and ML to MSW enhances the efficiency of vermicomposting and yields a nutrient-rich, value-added biofertilizer.*

1.0 Introduction

Considerable quantity of solid and liquid waste products generated from sewage units, paper factories, pulp industries, wastes from huge Indian horticulture sector and other domestic waste management units. The discarding of these wastes by conservative adopted technologies like open clearance and land filling claim soaring financial assets the methods reduce land dimensions for farming activities and is also perverse as it creates hurdles to expand vigorous and sanitary societies (Fatimah et al., 2020). Supervision and throwing away of these unnecessary industrialized wastes by methodological and specific scientific methods have become an exigent global question (Martin et al., 2021). Agricultural dispensation units and industries are producing mammoth quantity of plant nutrients. The production of 10.11 million tons of paper in India per year with an average increase of 2.6% of global paper production approximately produces 3.033 million tons of PMS per annum (Goel and Kalamdhad, 2017). To deal with these engineering and metropolitan solid wastes scientific and ecological responsive technologies have been brought into effect for supervision of these wastes at low input basis (Tang et al., 2020). The usual organic nutrients present in these wastes could be improved to raise soil lushness and to exchange the sterile land into nutrient rich soil, competent for agricultural use (Sharma et al., 2019).

Many epigeic earthworm species alleviate the diversity of organic residual materials which has been successfully done with the modification of microbial agents (Ravindran et al., 2020) and phosphate solubilising bacteria, Pseudomonas fluorescens (Lukashe et al., 2019) other than animal dung for nutrient rich vermicompost creation or vermicompost with disease-suppressive agents (Guimaraes et al., 2017). The apposite sustainable machinery to exploit the industrial slush as compared to incineration and land filling is the stabilization with earthworms and the bio-transformed end material (vermicompost) can be used as an agricultural soil conditioner (Mondal and Palit, 2019).

Earthworms were lucratively experimented to feed on various organic wastes such as: winery and distillery (Anawar et al., 2019); textile mills; power fly ash sugar processing (Karwal and Kaushik, 2020); oil and rice straw (Athira et al., 2019) and paper waste (Senthil and Sivakami, 2020). Vermicomposting is a novel method of vermiculture biotechnology to provide cost-effective technology for waste managing in a sustainable approach (Kakati et al., 2022). The dispensation rate of waste administration depends on the effectiveness of earthworm species and the nature of organic substrate used for vermicomposting apart from various other factors (Wijeysingha & Fernando, 2021). Vermicomposting generates a low- mass product with less processing time, humus content, product with less phyto-toxicity, high fertilizer value, increased N and an increased population of earthworms is also obtained for further use (Aslam et al., 2021). Vermicomposting develops a well-defined morphological change in the initial substrate and also processes the substrate in a way to ensure disappearance of fibres from the substrate (Orta et al., 2021).Vermicomposting is now regarded as more technological and technical routine for converting municipal solid waste and mango litter into a precious nutrient rich fertilizer at little input basis (Gaur et al., 2020).

In the present study, the administration and managing of MSW along with ML and CD has been estimated by using an epigeic earthworm Eisenia fetida species for vermicomposting. Finally, vermicomposting resulted in the creation of elevated nutritive value bio-fertilizer along with advantageous micro-organisms.

2.0 Materials and Methods

2.1 Municipal solid waste, Mango litter and culturing of earthworms

The MSW was collected from Omakulam dumping site Chidambaram, Cuddalore Tamil Nadu (India). It was collected in bulky gunney bags and was brought to the vermibiotech laboratory of Zoology Department Annamalai University Chidambaram, India. The collected municipal solid waste was tested to get the special physio-chemical features: 7.23 pH, 420.41 g kg–1organic C, 9.17 g kg–1 total N, 17.6 g kg–1 P, 4.2 g kg–1 K, and 15.9 C:N ratio. The MSW was shade dried for 2 days followed by treatment in hot air oven at 600C. The CD was collected from dairy farm of Annamalai University, faculty of agriculture, Chidambaram, India. The ML used was obtained from Department of Horticulture, faculty of agriculture Annamalai University, Chidambaram, India. The ML was shade dried and chopped prior to bedding of material for vermicomposting.

2.2 Experimental setup

The buckets having 32 cm diameter and 28 cm depth made of plastic, covered with nets for ventilation were utilized as vermicomposting units. The MSW, ML and CD were mixed in dissimilar ratios to develop 10 different treatments (Table 1). The combination of this organic material was used as food by earthworms and also served as the bedding material. The buckets containing the bedding material were accurately maintained with dissimilar substrates and the bucket with CD only was maintained as control.

The investigational buckets with bedding material were kept for three weeks for softening the organic waste by microbial degradation prior to investigation. From the culturing tanks, the earthworm Eisenia fetida with 28-week-old clitellate having individual live weight of 100±10 mg were taken and inoculated into different experimental buckets each containing 1 kg (dry weight) of substrate. After the inoculation of earthworms, the controlled moisture content of 70–80% throughout the study period of 60 days was maintained in the experimental containers by sprinkling of tap water. To control the loss of moisture due to high temperature the experimental buckets were covered with straw and nets. A well calculated humid and dark laboratory room

Table 1: Composition of substrate in vermibins used for experiment.

Treatment No.	Detail of treatments	Ratio of substrates	Quantity of wastes (Kg)			
			ML	MSW	CD	Total
T1	CD (control)	1:0	0	0	1	1
T2	ML+CD	1:1	0.5	0	0	1
T3	MSW+CD	1:1	0	0.5	0.5	1
T4	ML+MSW+CD	1:1:1	0.33	0.33	0.33	1
T5	ML+MSW	1:1	0.5	0.5	0	1
B1	ML+CD	2:1	0.75	0	0.25	1
B2	MSW+CD	2:1		0.75	0.25	1
B3	ML+MSW+CD	2:2:1	0.4	0.4	0.2	1
B4	ML+MSW	2:1	0.75	0.25	0	1
B5	ML+MSW	1:2	0.25	0.75	0	1

Mean values followed by different letters are statistically different (ANOVA,Tukey's t test P<0.05).

was maintained at a temperature of 32 ± 2ºC throughout the study phase. Three replications of samples were collected after each 20 days gap from each experimental bedding for analysis of various chemical changes such as: OC, N, P, K and C: N ratio throughout the period of 60 days. The total microbial population (bacteria, fungi and actinomycetes) and a variety of other parameters analyzed from the samples taken at the same interval of 20 days from different investigational treatments up to 60 days.

3.0 Results and Discussions

3.1 Physico-chemical characteristics

Table 2 and 3 represented the chemical changes that took place during vermicomposting. The vermicomposting process caused significant changes in the chemistry of these ten treatments, after 60 days of experimentation.

3.2 Changes in pH

As compared to the initial values (Table 2), pH decreased in B5 (18%) followed by T1 (15%), B1(13.2%), B4 (11.9%), B3 (8.7%), T5(8%), B2 (7.3%), T4 (7%), T2 (6%) and T3 (2%). This decline in pH is measured to be because of microbial activity, which results in the production of CO2 and organic acids during vermicomposting process and hence lowers the pH (Niedzialkoski et al., 2021) The accumulation of N and P into nitrates/nitrites and orthophosphates and changing of the organic material into transitional species of the organic acids is also considered to be the reason for the diminution in pH concentration (Nieder et al., 2018).

3.3 Changes in OC, N, P, K and C: N

The OC content was reduced in all the ten treatments. The loss in OC over the initial levels (Table 2) was higher in T1 (57.31%) treatment followed by B3 (35.75%), T2 (26.32%), B4 (23.77%), T3 (23.34%), B2 (21.25%), B1 (20%), T5 (15.91%), B5 (13.23%) and in T4 (12.5%). Earthworms endorse the crashing of organic matter and facilitate the microbial degradation of the substrate. The loss of carbon from the substrate takes place through microbial respiration in the form of CO_2 and also due to mineralization of OC due to the action of earthworms on the organic substrate. The microbial population is increased due to the production of mucus, ammonia, organic matter and body fluids by earthworms (Yuvaraj et al., 2020). The decline in OC and increase in the waste stabilization process is professionally done by earthworms (Kauser et al., 2021).

The total N content in vermicompost was amplified in all the substrate treatments over the initial values. As compared to the preliminary values, the highest rise in total N content recorded in different combinations of substrate was 26.31% in T1 followed by 24.77% in B1, 17.85% in T3, 16.66% in T2, 8.79% in B5, 8.60% in B2, 8.53% in T5, 8.33% in B3, 7.89% in T4 and 4.2% in B4 treatments (Table 3). Eisenia fetida adds the expensive content of N to the vermibeds by integrating its cast, mucous, enzymes, and growth stimulating hormones and also by the protein rich earthworm tissues after they die. The putrefaction of organic substrate by earthworms promote N mineralization process in the initial substrate, thereby enriches the N profile (Li et al., 2020). Earthworms mostly contain about 60-65% of protein in their tissues which is returned to the soil as N upon mineralization. Nearly 70% of the N in dead earthworm tissues gets mineralized within 20 days (Abail and Whalen, 2021). The quality of the substrate material defines the rotten activities and N mineralization by earthworms during vermicomposting.

Content of P in the substrate material also showed substantial boost upon vermicomposting. The maximum content of P increase was observed to be 72.72% in T5 treatment followed by 26.66% in B5, 25.58% in T1, 25.0% in T2, 24.13% in T3, 24.0% in B1, 23.52 in B3, 21.42% in B4, 19.51% in B3 and 18.18% in T4 (Table 3). The gut of the earthworm processes the organic Left-over material into the precious P content which is more available for the growth of plants. Phosphate solubilising bacteria in the casts supplementary assist the discharge of P. The accessible P formed in earthworm gut is mediated to plants by phosphatages. The microorganisms in the gut of earthworm facilitate in the metabolism of P by the action of gut enzymes.

Vermicomposting of organic bits and pieces also Increases the K concentration in different substrate treatments, the highest K content among the different treatments was observed to be 60.75% in B5, followed by 50.0% in T5, 41.17% in B4, 36.92% in T2, 35.59% in B1, 35.29% in B3, 33.11% in T1, 30.15% in T4, 27.83% in B2 and 23.65% in T3 after 60 days (Tables 2).

Table 2. Initial chemical composition (g kg^{-1}) of different treatments used for experiment (mean±SD).

Treatment	PH	Organic carbon	Total Nitrogen	Available P	Exchange-able K	C:N ratio
T1	8.50±0.10	164.73±1.20	0.95±0.02	0.43±0.01	6.11±1.10	39.30±2.50
T2	7.48±0.20	190.21±2.30	1.70±0.04	1.20±0.30	6.53±1.50	31.00±2.40
T3	8.00±0.23	300.32±2.00	8.40±0.52	2.90±0.12	9.30±2.50	23.40±3.00
T4	8.20±0.32	400.43±1.10	11.40±0.23	3.30±0.11	12.64±2.40	22.10±1.50
T5	8.10±0.21	220.90±1.00	0.20±0.04	2.20±0.40	7.42±2.60	29.30±1.10
B1	7.53±0.22	200.50±3.40	1.13±0.30	2.50±0.21	5.90±2.50	38.50±2.40
B2	8.03±0.21	320.45±1.00	9.30±0.50	3.40±0.33	9.70±2.60	30.20±3.20
B3	8.40±0.35	481.60+2.00	17.00±0.6	4.10±0.34	13.60±1.40	19.50±3.10
B4	7.39±0.21	260.32+3.50	7.00±0.30	2.80±0.45	6.84±1.80	20.90±1.80
B5	8.30±0.11	310.75±2.30	9.10±0.15	3.00±0.30	7.90±1.50	27.30±2.00

Mean values followed by different letters are statistically different (ANOVA, Tukey's t test P<0.05).

Therefore during Vermicomposting the combined accomplishment of micro-organisms and earthworms on organic materials result in the release of plant nutrients. In the present examination it was revealed that K content increased over the initial values by vermicomposting.

Vermicomposting also reduced the C: N ratio in the organic substrate, the highest diminution in C: N ratio was observed to be 30.34% in T3 followed by 27.18% in B3, 21.16% in T5, 20.88% in B5, 20.78% in B1, 17.19% in T4, 16.03% in T1, 12.90% in T2, 11.08% in B4 and 10.26% in B2 (Table 3). The respiratory activities of earthworms and micro-organisms reduces the C content in the initial substrate to that of the final compost due to the loss of carbon dioxide while the increase in N content in the substrate is due to the production of mucus, enzymes and nitrogen rich excreta by earthworms that lowers the C: N ratio (Sharma and Garg, 2018).

3.4 Quantitative analysis of Microbes

The total microbial populations (bacteria, fungi and actinomycetes) in different MSW, ML and CD mixtures acted upon by Eisenia fetida at different time intervals (0, 20th, 40th and 60th day) are presented in Tables, 4, 5 and 6.

Table 3. Chemical composition of different substrates at the end of experiment (vermicomposting).

Treatment	PH	Organic carbon	Total Nitrogen	Available P	Exchange-able K	C:N ratio
T1	7.20±0.10	70±1.4	1.2±0.32	0.54±.01	8.12±0.81	33.01±2.11
T2	7.48±0.31	140±1.7	1.4±0.57	1.5±0.11	8.9±0.64	27.32±1.32
T3	7.80±0.10	230±2.6	9.9±1.22	3.3±0.31	11.5±1.43	16.55±1.43
T4	7.50±0.52	350±3.0	12.3±1.83	3.9±0.22	16.4±1.23	18.3±1.10
T5	7.30±0.22	185±3.5	8.9±0.75	2.7±0.50	11.1±1.14	23.4±2.15
B1	6.20±0.40	160±1.4	1.41±0.60	3.1±0.23	8.0±0.90	30.5±2.50
B2	7.30±0.61	252±1.8	10.1±1.55	4.2±0.63	2.4±0.45	27.1±2.10
B3	7.50±0.23	309±2.3	13.0±0.74	4.9±0.65	18.4±1.50	14.2±1.22
B4	6.20±0.15	200±2.1	7.3±0.90	3.4±0.28	9.6±1.10	15.4±1.35
B5	6.50±0.36	269±3.2	9.9±1.88	3.8±0.21	12.7±1.12	21.6±1.85

Mean values followed by different letters are statistically different (ANOVA, Tukey's t test P<0.05).

Quantitative investigation of micro-organisms was done by serial dilution method and standard pour plate methods. The number of colony forming Units (CFU) was expressed as CFU g1. The samples for microbial analysis were collected from control and other several combinations of substrate treatments. The microbial populations (bacteria, fungi and actinomycetes) were investigated from the samples taken on the 0th, 20th, 40th and 60th days of experimental period by the following methods.

3.5. Total number of bacterial population (CFU × $10^6 g^{-1}$)

In the present study investigation revealed that the bacterial population increased significantly ($p<0.05$) in control and other substrate treatments. The bacterial population increased gradually in all the combinations of substrate treatments (Table 4).

The bacterial population increased gradually in Eisenia fetida treated experimental media (Table 4) over the initial values throughout the study period of 60 days. The highest bacterial population in the vermicompost was obtained in T1 (610.12±1.83 in CFU×10^6) followed by T2 (520.15±2.33 in CFU×10^6), T3 (490.20±2.08 in CFU×106), B1 (430.57±1.95 in CFU×10^6), T4

(426.67±1.92 in CFU×10^6), B3 (425.95±1.88 in CFU×10^6), B2 (415.23±1.32 in CFU×10^6), T5 (395.12±2.31 in CFU×10^6), B4 (319.21±1.42 in CFU×10^6) and B5 (331.15±2.50 in CFU×10^6) on 60th day. The percentage difference in bacterial population over the initial values recorded at the end of the experimental period of 60 days were T5 (83.72%), B2 (64.68%), B4 (59.5%), B3 (54.54%), T1 (52.5%),T3 (49.39%), B5 (44.54%), T2 (44.44%), T4 (42.95%) and B1 (40.06%) respectively.

Table 4. Bacterial population (CFU × $10^6 g^{-1}$) in vermicompost at different time intervals.

Treatment	0 days	20 days	40 days	60 days
T1	400.22±2.11	450.23±1.80	510.12±1.12	610.12±1.83
T2	360.15±1.50	390.50±2.12	442.14±1.22	520.15±2.33
T3	328.44±1.80	367.45±1.92	425.45±1.53	490.20±2.08
T4	298.36±1.32	320.15±2.08	380.25±1.20	426.67±1.92
T5	215.60±1.45	249.15±1.32	312.40±1.60	395.12±2.31
B1	307.46±1.88	329.35±1.42	385.15±1.55	430.57±1.95
B2	252.23±2.15	292.80±2.25	350.45±1.65	415.23±1.32
B3	275.21±2.54	298.68±1.88	360.25±1.48	425.95±1.88
B4	200.30±2.45	225.95±1.15	275.12±1.28	319.21±1.42
B5	229.45±2.10	247.25±1.45	285.24±1.68	331.15±2.50

**Results are reported as mean ± standard deviation; mean values followed by different letters are statistically.*

3.6 Total number of fungal population (CFU×10^4 g^{-1})

The present study investigated that the fungal population increased significantly ($p<0.05$) over the initial values in all the substrate treatments (Table 5). The highest number of fungal colonies were observed in the end product obtained from T1 (269.14±0.83 in CFU×104) followed by T2 (23.15±1.13 in CFU×10^4), T3 (203.05±1.18 in CFU×10^4), T4 (195.17±1.12 in CFU×10^4), B2 (187.07±0.95 in CFU×10^4), B5 (181.10±1.50 in CFU×10^4), T5 (162.13±1.02 in CFU×10^4), B3 (161.15±1.12 in CFU×10^4), B1 (147.10±1.25 in CFU×10^4) and B4 (123.11±1.70 in CFU×104) on 60th day. The percentage change in the population of fungi recorded at the end of the study period of 60 days over the initial values are ranked as (96.35%) in T1, (89.34%) in T2, (89.32%) in T4, (81.48%) in B1, (81.25%) in T3, (79.20% in B5, (74.76%) in B2, (70.52%) in T5, (57.69%) in B4 and (40.0%) in B3 repectively.

Table 5. Fungal population (CFU×$10^4 g^{-1}$) in vermicompost at different time intervals.

Treatment	0 days	20 days	40 days	60 days
T1	137.40±1.11	14.129±0.55	230.12±1.50	269.14±0.83
T2	122.75±0.85	165.11±0.38	189.15±1.40	231.15±1.13
T3	112.25±0.90	137.80±0.45	160.25±0.95	203.05±1.18
T4	103.50±0.70	121.85±0.25	157.40±1.15	195.17±1.12
T5	95.60±0.50	107.60±0.49	131.90±1.76	162.13±1.02
B1	81.54±0.60	101.80±0.90	114.50±1.90	147.10±1.25
B2	107.89±0.45	129.54±0.85	165.80±1.83	187.07±0.95
B3	115.74±0.95	126.76±1.22	142.70±0.75	161.15±1.12
B4	78.20±0.15	97.35±0.50	113.50±0.55	123.11±1.70
B5	101.75±0.45	124.40±0.35	154.45±0.80	181.10±1.50

**Results are reported as mean ± standard deviation; mean values followed by different letters are statistically different (Tukey's t test P<0.05).*

3.7 Total number of Actinomycetes (CFU×$10^5 g^{-1}$)

The population of actinomycetes in vermicompost produced from MSW mixed with ML and CD in different ratios used by E. fetida is illustrated in Table 6.

The population of actinomycetes in different substrate combinations was recorded to be highest in B3 (26.12±0.13 in CFU × 105) followed by T1 (26.11±1.00 in CFU × 105), T4 (25.10±0.95 in CFU × 105), T2 (24.50±0.50 in CFU × 105), B2 (22.05±0.53 in CFU × 105), T3 (21.40±0.82 in CFU ×105), T5 (21.00±0.15 in CFU×105), B5 (19.60±0.95 in CFU×105), B1 (17.50±0.25 in CFU× 105) and B4 (16.20±1.20 in CFU× 105) on 60th day (Table 6). The percentage change in actinomycetes population recorded on 60th day over the initial values were (90.36%) in B4, (86.17%) in B1, (84.91%) in T1, (70.43%) in B5, (69.59%) in T4, (65.61%) in T5, (64.42%) in T2, (56.20%) in T3, (50.98%) in B3 and (46.02%) in B2 respectively.

Table 6. Actinomycetes population (CFU×$10^5 g^{-1}$) in

vermicompost at different time intervals.

Treatment	0 days	20 days	40 days	60 days
T1	14.12±1.22	20.18±1.22	23.20±1.15	26.11±1.00
T2	14.90±1.33	17.10±1.10	18.90±1.40	24.50±0.50
T3	13.70±1.35	14.90±1.75	17.51±1.50	21.40±0.82
T4	14.80±1.50	17.50±1.64	21.51±1.45	25.10±0.95
T5	12.68±1.80	15.40±1.43	16.10±1.20	21.00±0.15
B1	9.40±1.65	11.50±1.80	13.90±1.56	17.50±0.25
B2	15.10±1.30	17.90±1.30	19.31±1.25	22.05±0.53
B3	17.30±1.25	22.04±1.11	20.30±1.49	26.12±0.13
B4	8.51±1.20	10.60±1.25	13.40±1.60	16.20±1.20
B5	11.50±1.75	13.30±1.55	17.00±1.99	19.60±0.95

**Results are reported as mean ± standard deviation; mean values followed by different letters are statistically different (Tukey's t test P<0.05).*

4.0 Conclusions

Treatment of municipal solid waste with Eisenia fetida not only enriched the substrate with useful nutrients for plants. Eisenia fetida was found to be a potential animal for conversion and management of Municipal solid waste. The waste when combined with ML and CD proved to be a good growth media for the earthworm. Vermicomposting has been found to be substantially appropriate for the growth of valuable microbes that encourage the alteration of organic substances into a high energy, humus rich content for sustainable plant and crop production. Finally, the investigation has fulfilled our ultimate aim of municipal solid waste management scientifically.

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Declaration of conflict of interest

The authors have collectively contributed to the conceptualization, design, and execution of this journal. They have worked on drafting and critically revising the article to include significant intellectual content. This manuscript has not been previously submitted or reviewed by any other journal or publishing platform. Additionally, the authors do not have any affiliation with any organization that has a direct or indirect financial stake in the subject matter discussed in this manuscript.

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