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Bio-conversion of pre-treated agricultural waste to compost and vermicompost

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Abstract
Compost is a mixture of organic residues contain animal dung and urine along with other residues, such as fodder ruminant, stubble, weeds and leaves. Therefore the investigated study was designed to evaluate the maize straw to integrated management of composting and vermicomposting. The experimental trial was carried out at Agricultural college, Rajendranagar, Hyderabad. Cellulose degrading microbes were isolated from different soil samples, efficient cellulose-degrading microorganisms were identified, the outstanding isolates were used for the pretreatment of agricultural waste (maize straw). The elevated temperatures found during the thermophilic phase are essential for rapid degradation of lignocellulose. Vermicomposting results in significantly decreased in pH, Total organic carbon (TOC), electrical conductivity (EC) and C: N ratio while a significant increase in total Kjeldahl nitrogen (TKN) available phosphorus, exchangeable potassium and calcium in vermicomposts/vermiwash. This study clearly indicates that vermicomposting of animal, agro/kitchen wastes not only produced a valuable vermicompost/vermiwash but also increased the level of plant growth supplements in final vermicompost.

Keywords: Cellulose, lignocellulose, agricultural waste, horticultural waste, thermophilic

1. Introduction
The excess uses of chemical fertilizers and pesticides have made our soil sick and problematic and cause environmental hazards which affect human health and the environment. Million of tons of animal, agro and kitchen wastes are produced annually and have odor and pollution problems (Suthar *et al*., 2005; Reinecke *et al*., 1992; Garg *et al*., 2006) [15, 12, 7]. Much attention has been paid in recently passed years to manage different organic wastes resources at low input as a well eco-friendly basis. Vermicomposting, through earthworms, is an Eco biotechnological process that transforms energy-rich and complex organic substances into a stabilized vermicomposts (Bentize *et al*., 2000) [5]. Composting is the conversion, by microorganisms, of complex mixtures of quickly degradable organic materials to more stable, humified materials, usually in a warm, moist, and relatively aerobic environment. The borderline between waste and compost has not been precisely defined by generally agreed upon quantitative parameters, but it is assumed that composts should not reheat anaerobically during curing and storage, producing foul odors. Composting satisfies the health and aesthetic aspects of waste disposal by destroying almost all pathogens. In addition, the product is agriculturally or horticulturally beneficial as a soil conditioner and fertilizer. Much of the research on composting has focused on the changes in the physical and chemical parameters of the compost, primarily in an effort to find a simple and reliable indicator of compost maturity and to improve the efficiency of the process. However, it is important to remember that the compost microbiota determines the rate of composting and the quality of the product. Their actions will depend upon the nutrients available and the physical parameters surrounding them during the process. The purpose of this study was to provide a better understanding of the physical and chemical factors which may influence microbial activity in composting and vermicomposting of agricultural waste. The moisture content, pH, organic content, total nitrogen, total carbon, total protein, and temperature were examined at various stages of the process with regard to the microbial biomass and activity in the compost and vermicompost. Although many of these parameters have previously been measured in composts, few data have been collected which would allow simultaneous correlations between all of these parameters.

2. Materials and Methods

2.1 Bacterial Strains Isolation

Cellulolytic bacterial strains were isolated from soil by using serial dilutions and pour plate technique. The medium used for isolation of cellulolytic bacteria contains 1.0 % peptone, 1.0 % carboxymethyl cellulose (CMC), 0.2 % K_2HPO_4, 1 % agar, 0.03 % $MgSO_4.7H_2O$, 0.25 % $(NH_4)_2SO_4$ and 0.2 % gelatin at pH 7 for 48 hours of incubation at 30 ºC (Yin *et al.,* 2010). Bacterial colonies were purified by repeated streaking. The purified colonies were preserved at 4 ºC for further identification and screening for cellulase production.

2.2 Screening of cellulolytic bacteria

Pure cultures of bacterial isolates were individually transferred in CMC agar plates. After incubation for 48 hours, CMC agar plates were flooded with 1 % colored and allowed to stand for 15 min at room temperature. One molar NaCl was thoroughly used for counterstaining the plates. Clear zones were appeared around growing bacterial colonies indicating cellulose hydrolysis (Andro *et al.,* 1984) [1]. The bacterial colonies having the largest clear zone were selected for identification and cellulase production in the submerged system. The formation of a clear zone of hydrolysis indicated cellulose degradation. The ratio of the clear zone diameter to colony diameter was measured in order to select for the highest cellulase activity producer. The largest ratio was assumed to contain the highest activity.

2.3 Identification of cellulolytic bacteria

Identification of cellulolytic bacteria was carried out by method as described by Cowen and Steel (Barrow and Feltham, 1993) [3] and (Cullimore, 2000) [6]. The bacterial isolates were presumptively identified by means of morphological examination and some biochemical characterization. The parameters investigated included colony characteristics, shape, size, spore, motility, Gram's reaction, catalase production, urease production, Voges-Proskauer (V-P) reaction, Indole production, Nitrate reduction, citrate utilization, carbohydrate metabolism (acid-gas production), starch hydrolysis.

2.4 Inoculum development

Pure cultures of selected bacterial isolates were individually maintained on CMC supplemented minimal agar slants at 4^0 C until used. Pure cultures of selected bacterial isolates were inoculated in a broth medium containing 0.03 % $MgSO_4$, 0.2 % K_2HPO_4, 1 % glucose, 0.25 % $(NH_4)_2SO_4$ and 1 % peptone at pH 7 for 24 h of the fermentation period. After 24 h of the fermentation period, these vegetative cells were used as inoculum source (Basavaraj *et al*. 2014) [4].

2.5 Cellulase activity of the isolates

For the quantitative estimation of cellulase, the bacterial isolates were grown in carboxymethyl cellulose broth. Cellulase activity was assayed using dinitrosalicylic acid (DNS) reagent (Miller, 1959) [9] by estimation of reducing sugars released from CMC solubilized in 0.05 M phosphate buffer at pH 8 (Bailey *et al.,* 1992) [2]. The culture broth was centrifuged at 14000×g for 10 min at 4^0 C and the clear supernatant served as crude enzyme source. The crude enzyme was added to 0.5 ml of 1 % CMC in 0.05 M phosphate buffer and incubated at 50 ºC for 30 min. After incubation, the reaction was stopped by the addition of 1.5 ml of DNS reagent and boiled at 100 ºC in a water bath for 10 min. Sugars liberated were determined by measuring absorbance at 540 nm. Cellulase production was estimated by using a glucose calibration curve (Shoham *et al.,* 1999) [13]. One unit (U) of enzyme activity is expressed as the quantity of enzyme, which is required to release 1µmol of glucose per minute under standard assay conditions.

2.6 Maintenance of the efficient isolates

Bacterial isolates selected by the above method were maintained on nutrient agar plates for routine use and stored at 4 °C. Bacterial isolates were transferred into fresh plats after every 15 days interval.

2.7 Procedure for pretreatment

First, 1 g dry ground corn straw powder was poured into a 118 ml serum glass bottle, after which 10 ml distilled water was added into the bottle. After autoclaving for 120 min, the bottles were inoculated with the complex microbial agents in aseptic conditions. A 0.01 % (w/w) dose of the complex microbial agents was used in our study. Each of 20 bottles was then covered and sealed with a plastic film. A sponge plug was inserted into the middle of the film to sparge air while preventing airborne microorganisms from entering the bottle. The control bottle was not inoculated with complex microbial agents. It only contained 1 g dry ground corn straw and 10 ml distilled water. The bottles were placed in an incubation chamber. Samples were obtained on the 0th, 5th, 10th, 15th, and 20th day of incubation for composition determination, chemical analyses and biochemical methane potential (BMP) assay (Prasanna *et al*. 2016) [10].

2.8 Preparations of soil beds

The experiment was conducted as per the method adopted by (Yasmin and D'Souza, 2007) [16]. Plastic tubs were used for preparations of soil beds for the earthworm. Dried soil (from nearby farmland) was crushed and filtered through a fine mesh sieve. The weighed fine soil was then poured in each plastic tub and water was added to moistened the soil, then 500 gm dried powdered cow dung (3 weeks old) was also added to each plastic tub to avoid starvation thus maintaining soil: cow dung ratio of 1:1.

2.9 Experimental set-up

20 mature earthworms were added to each plastic tub of different dose treatment of the fertilizers in addition to the control set. Thus one control set and four experimental sets were prepared. Three replicates were used for each set to get an average value of each parameter under study. To maintain up-to 70 percent moisture level, water was supplied regularly till the end of the experiment. The tubs were covered with a wet muslin cloth so that the essential moisture level needed by the worms is maintained and also it prevented them to crawl out of the tub. By the end of 60 days, the soil samples were drawn for analysis from each of the experiment tubs excluding the earthworms and their cocoons and juveniles (Priyanka *et al*, 2015) [11].

2.10 Determination of soil pH

For determination of pH, soil suspension in the ratio of 1:5 was prepared. The suspension was stirred at regular intervals for 30 minutes and the soil pH was recorded by the digital pH meter (Jackson, 1962) [8].

2.11 Determination of soil available nitrogen, phosphorus and potassium

For determining available nitrogen in soil sample method described by Subbiah and Asija, (1956) [14] was adopted.

3. Results and Discussion

3.1 Isolation screening of cellulose degrading bacteria

In the present study, six different sources of samples were chosen for the isolation of efficient cellulolytic bacterial isolates. Among six different sources, in SWS sample 155 CFU isolates were recorded followed by other sources HDD (145 CFU), FF (110 CFU), MSW (97 CFU), CDS (64 CFU) and DKW (52 CFU) are recorded. Overall fifteen cellulolytic bacteria were isolated from soil samples. These fifteen bacteria were screened for cellulase production in a submerged fermentation process using a suitable medium. Among all these fifteen tested bacterial strains; some strains gave better yield as shown in Table 2. Fresh culture of this isolate consists of Gram-negative, slender and rod-shaped cells but the older cultures contain coccoid cells. Microscopic examination of this isolate revealed that it was gram negative, non-spore forming and motile. It was negative for indole production, Voges Proskauer test and citrate utilization and positive for catalase and nitrate reduction. It could ferment glucose, cellulose, lactose and sucrose.

Table 1: Isolation of cellulose degrading bacteria

Samples	(10^6 CFU g^{-1})
CDS	64
MSW	97
DKW	52
SWS	155
FF	110
HDD	145
C.D. (P=0.05)	1.799
SE(m)	0.577
C.V.	0.965

CDS- Cow dung from cattle shed at Rajendranagar; MSW- Municipal solid waste from garbage disposal place of Rajendranagar; DKW- Domestic kitchen waste from hostel mess; SWS- Sewage water sample from sewage sludge of Rajendranagar; FF-Farmers' Fields of college farm at Rajendranagar; HDD-Horse dung dump near the veterinary college.

CCRA- Control plate CCRA- Media plate with cellulose-degrading bacterial colonies

Plate 1: Isolation of cellulose degrading bacterial isolates

Table 2: Cellulase activity of bacterial isolates

S. No	Isolates	CDS	MSW	DKW	SWS	Zone (mm)	Enzyme activity (mg/ml)
1	CDB-1	+	-	-	-	0.75	0.032
2	CDB-4	-	+	-	-	0.35	0.020
3	CDB-5	-	+	-	-	0.20	0.016
4	CDB-7	-	-	+	-	0.55	0.015
5	CDB-9	-	-	+	-	0.53	0.015
6	CDB-10	-	-	-	+	1.25	0.136
7	CDB-11	-	-	-	+	1.18	0.126
8	CDB-15	-	-	-	+	1.13	0.124
9	CDB-18	-	-	-	-	0.25	0.018
10	CDB-21	-	+	-	-	0.98	0.019
11	CDB-23	-	+	-	-	0.75	0.020
12	CDB-24	-	-	+	-	0.93	0.013
13	CDB-26	-	-	+	-	1.18	0.125
14	CDB-28	-	-	-	+	0.98	0.024
15	CDB-30	-	-	-	+	1.38	0.149
	C.D.					0.058	0.005
	SE(m)					0.020	0.002
	C.V.					4.225	5.493

CDS-Cow dung sample; MSW- Municipal solid waste; DKW- Domestic kitchen waste; SWS- Sewage water sample. + and – indicate: Source of sample for isolation of bacterial isolates

Table 3: Biochemical characteristics of cellulose degrading bacterial isolates

S. No	Biochemical tests	CDB														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	Indole test	-	-	+	-	+	+	-	-	-	-	-	-	-	-	-
2	Catalase test	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+
3	Oxidase test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4	Gelatin liquifaction	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
5	Methyl red test	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
6	V-P test	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+
7	Citraye utilization	-	+	-	+	+	+	+	+	+	-	+	+	+	-	+
8	Starch hydrolysis	-	+	-	+	+	-	-	-	-	-	-	-	-	-	+
9	H_2S	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+
10	Denitrification	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
11	Glucose	+	-	-	+	+	+	-	+	-	+	+	+	+	+	+
12	Galactose	-	-	+	+	-	+	-	-	+	+	-	+	-	-	-
13	Lactose	-	+	-	+	+	-	-	+	-	+	+	-	-	+	+

Table 4: pH of different treatments

	pH		
	Initial	14th Day	28th Day
T_1	8.00	8.25	8.18
T_2	8.20	8.38	8.26
T_3	8.25	8.40	8.33
T_4	8.20	8.36	8.28

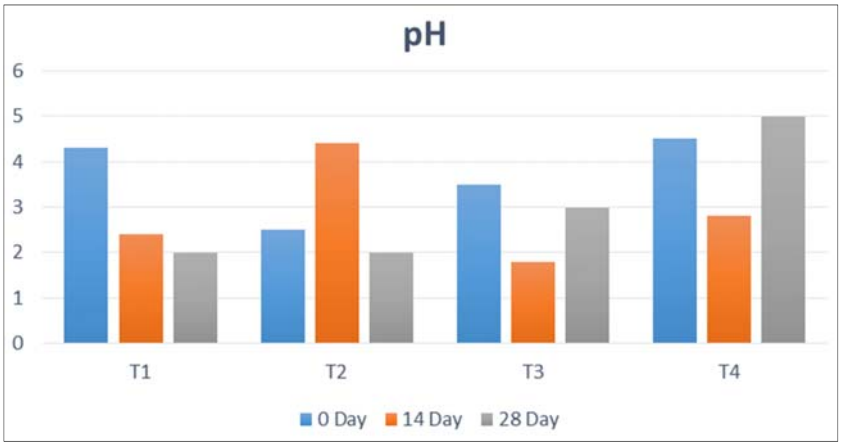
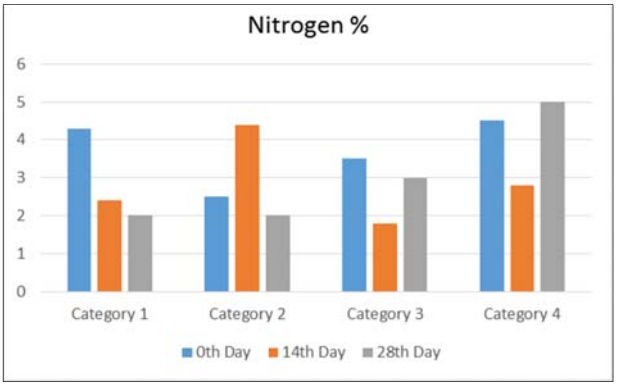
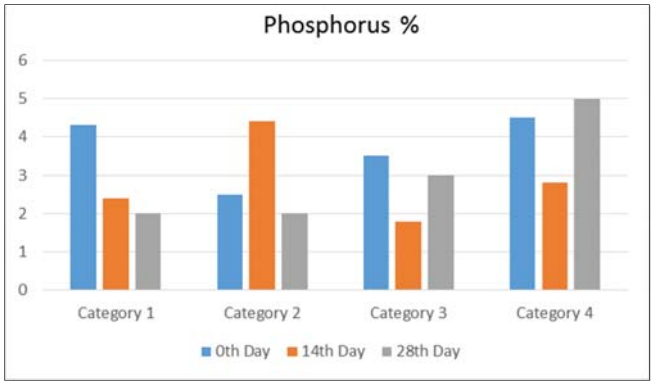
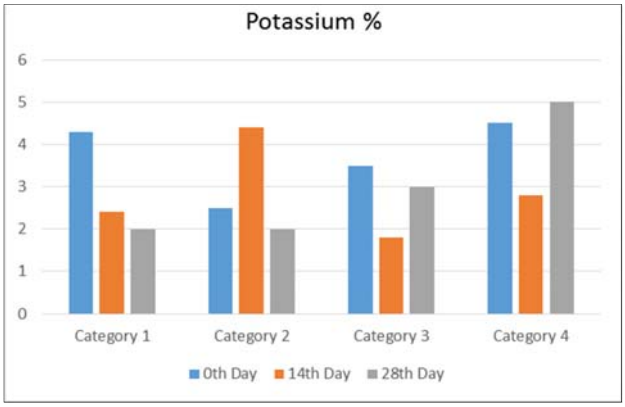


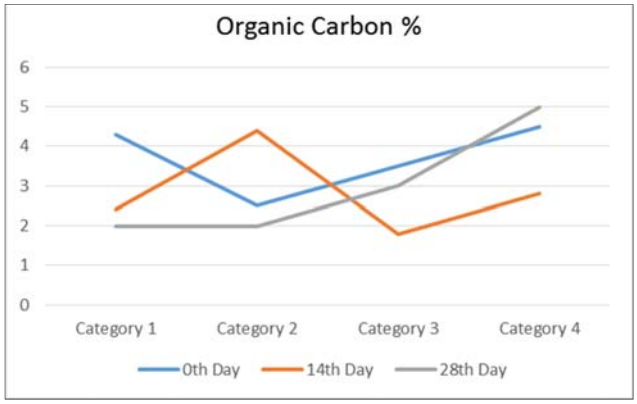
Table 5: Nutrient status of different treatments at different intervals of experiment

	Nitrogen (%)			Phosphorus (%)			Potassium (%)			Organic carbon (%)		
	Initial	14th Day	28th Day	Initial	14th Day	28th Day	Initial	14th Day	28th Day	Initial	14th Day	28th Day
T_1	0.60	0.65	0.70	0.15	0.24	0.17	0.65	0.71	0.72	25.50	28.15	26.70
T_2	0.70	0.79	0.90	0.16	0.25	0.19	0.70	0.76	0.78	24.00	26.78	25.00
T_3	1.65	0.84	1.91	1.00	1.28	1.06	0.99	1.15	1.01	21.60	24.50	22.00
T_4	1.84	1.86	2.41	1.00	1.26	1.10	1.00	1.26	1.05	22.60	26.48	23.50









T_1 - Composting without pretreatment, T_2 - Composting with pretreatment
T_3 - Vermicomposting without pretreatment, T_4 - Vermicomposting with pretreatment

3.2 Cellulase activity of bacterial isolates

Maximum clearing zone ranged from 1.38 and 1.25 mm demonstrating that the isolates have the ability to degrade the carboxymethyl cellulose and indicating the high ability of cellulase production. Among 15 bacterial isolates, the isolates CDB-30 as evidenced by its maximum clearing zone value of 1.38 mm, followed by CDB-10 (1.25 mm), CDB-11, CDB-26, CDB-15 (1.13 mm). Similarly quantitative estimation of cellulose activity results revealed that same manner (CDB-30: 0.149 mg ml^{-1}, CDB-10: 0.136 mg ml^{-1}) (Table 2).

3.3 Biochemical characteristics of cellulose degrading bacterial isolates

After the morphological characterization, the isolates were tested for different biochemical characters like catalase and urease production, IMVIC (Indole test, Methyl red reaction, Voges-Proskauer reaction, Citrate utilization), Nitrate reduction, Carbohydrate fermentation (acid-gas production), Starch hydrolysis, Gelatin liquefaction and Casein hydrolysis (Table 3). The biochemical test was conducted for all 15 bacterial isolates 3 isolates were positive for indole reaction, 12 isolates positive catalase activity, all isolates were positive for oxidase activity, Voges Proskauer test and Denitrification tests. 13 isolates were positive for gelatine liquefaction, 11 isolates were positive for citrate utilization, 4 isolates were positive for starch hydrolysis, 14 isolates were positive for H_2S production, and in carbohydrate utilization tests 11 isolates utilized glucose, 6 isolates utilized galactose and 7 isolates used lactose. All isolates exhibited denitrification activity. The isolates were identified based on gram reaction and colony morphology on different media. Fifteen isolates showed growth on specialized media. Observations recorded after streaking and incubation on a different medium. Both morphological, cultural biochemical characters were used for identification of the isolates using the criteria mentioned in Bergey's Manual of Systematic Bacteriology.

3.4 pH of different treatments

Among the different treatments the pH was found highest in T_3 (Vermicomposting without pretreatment) (8.25) on the 0^{th} day and the result was found on par with the treatments of T_2 (Composting with pretreatment), T_4 (Vermicomposting with pretreatment) and T_1 (Composting without pretreatment). Among the different intervals, the pH was slightly low initially and it was increased continuously up to 28^{th} day and slightly reduced in other treatments in the respective intervals.

3.5 Nutrient Status of Different Treatments At Different Intervals of Experiment.

Nitrogen content during pretreatment of agricultural waste found on par at different intervals in all the treatments except T_3 (Vermicomposting without pretreatment) (i.e. 0.84 to 1.65 %). Among all the intervals the N content gradually increased from 0^{th} day to 28^{th} day. Among the treatments, the N content was found significantly highest with the treatment T_4 (Vermicomposting with pretreatment) (i.e. 1.81 to 2.41 %) on the 28^{th} day followed by T_3 (Vermicomposting without pretreatment). Lowest N content was observed in T_1 (Composting without pretreatment) (Table 5). There was a significant variation in available nitrogen content in substrates between different treatments. This variation in available nitrogen content of substrates was noticed in all the stages of composting, vermicomposting and biogas production period. P content was found on par at different intervals in all the treatments. Among all the treatments P content was found significantly highest in T_3 (Vermicomposting without pretreatment) and T_4 (Vermicomposting with pretreatment) and least was found in the treatment T_1 (Composting without pretreatment). Among all the intervals P content was increased up to the 14^{th} day of pretreatment and the gradual reduction was observed up to the 28^{th} day (Table 5). It is evident from this experiment that increases in phosphatase activity by microorganisms leads to an increase in the amount of phosphorus which supports the phosphate availability in the substrates. Similarly the available potassium was on par from treatments of T_4 (Composting without pretreatment) on 0^{th} day (i.e. 1.00 to 1.00 %) And among all the treatments T_4 (Vermicomposting with pretreatment) (i.e. 1.00 to 1.26 %) showed the highest K content was observed with treatment T_1 (Composting without pretreatment) on 28_{th} day. Among all the intervals the K content gradually increased from 0^{th} day to 28^{th} day in T_2 (Composting with pretreatment) (Table 5). This could be attributed to the fact that with the passage of time the substrate composition changes and becomes suitable for microorganisms to work upon, in turn increases the activity of potassium in substrates between different intervals.

4. Discussion

The pH of both vermicompost and compost was found to be in the range of 8.25-8.00 initially and dropped to 8.14 during the process. The slight change in pH from slightly acidic to neutral is due to an increase in N, P and K content or Organic matter content. The results found that the pH of the substrate has a significant effect on composting, vermicomposting and biogas production because it affects the activity of bacteria to

degrade organic matter into biogas. A low pH in the digester inhibits the activity of microorganisms involved in the digestion process, particularly methanogenic bacteria. The breakdown of organic matter during the composting process is dependent on several factors working in concert. These include moisture, microbial populations, Oxygen (O_2), and a balance of Carbon (C) and Nitrogen (N). Microorganisms in the organic matter (OM) consume the readily available carbon. As it is metabolized, temperatures increase in the compost pile and Carbon dioxide (CO2) is released. As a result, the pile is newly populated with thermophilic, or heat-loving, bacteria that consume the rest of the degradable carbon. As microbial activity slows, temperature decreases, allowing for colonization by fungi that slowly consume much of the remaining recalcitrant forms of lignins and cellulose. The resulting crumbly, earthy humus is considerably more stable than manure, meaning that its nutrients are less likely to be lost to leaching or volatilization into the atmosphere. Nitrogen losses impact negatively on the manure composting process, by decreasing nutrient concentration and hence compost quality and generate health and environmental problems. Nitrogen losses through composting can occur by NH3-volatilisation, leaching and denitrification. Denitrification can occur as a result of the development of anaerobic microsites within the material. Thus, the aerobic conditions of the compost should be ensured throughout the process, indicated that emission rates of N2O–N were very much lower (about 10 times) than those of NH3–N during composting of cattle manure with maize straw.

5. Conclusion

Biological pretreatment with complex microbial agents proved to be an efficient method to improve biodegradability to enhance composting, vermicomposting of agricultural waste and horticultural waste. Compared to untreated controls the pretreated agricultural and horticultural waste yielded higher manurial value. Substrates were used for compost, vermicompost making in lab scale. In the present study, results revealed that agricultural and horticultural wastes pretreatment with efficient microbes helped aerobic composting, vermicomposting. By the pretreatment of agricultural waste, horticultural waste was easily degraded by enriched cultures and their enzyme activities. In the present study vermicompost with microbial pretreatment enhanced degradation and nutrient values compared to regular composting methods. Compared to aerobic composting, vermicomposting showed to be better. The N, P and organic carbon % increased in all the treatments of horticultural waste compared to agricultural waste. Considering the characteristics of the high moisture solid waste of agricultural and horticultural waste.

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6. References

1. Andro T, Chambost JP, Kotoujansky A, Cattaneo J, Bertheau Y, Barras F *et al*. Mutants of plant cells by pectic enzymes: the lack of direct injurious interaction between pectic enzymes or their soluble reaction products and plant cells. Phytopathology, 1984, 141-153.
2. Bailey MJ, Biely P, Poutanen K. Interlaboratory Testing of Methods for Assay of Xylanase Activity. Journal of Biotechnology. 1992; 23(3):257-270.
3. Barrow GI, Feltham RKA. Eds. Cowan and Steel's Manual for the Identification of Medical Bacteria. 3rd Edition, Cambridge University Press, Cambridge, 1993, 331.
4. Basavaraj IP, Shivasharan CT, Kaliwal BB. Isolation and Characterization of Cellulase producing bacteria from Soil. International Journal of Current Microbiology and Applied Sciences. 2014; 3(5):59-69.
5. Bentize E, Nogales R, Masciandro G, Ceccanthi B. Isolation by isoelectric focusing of humic urease complex from earthworm Eisenia foetida processed sewage sludge. Biology and Fertility of Soils. 2000; 31:489-493.
6. Cullimore DR. Practical atlas for bacteria identification. [M]. USA: Lewis Publishers. 2000.
7. Garg VK, Suthar S, Yadav A. Management of food industry waste employing vermicomposting technology. Bioresource Technology. 2006; 126:437-443.
8. Jackson ML. Soil chemical analysis. Prentice Hall. Englewood Cliffs, New York, U.S.A, 1962.
9. Miller. Use of dinitrosalicylic acid reagent for detection of reducing sugars. Analytical Chemistry. 1959; 31(3):426-428.
10. Prasanna KB, Triveni S, Subhash Reddy R, Vijaya Gopal A. Effect of Cellulolytic Pretreatment of Agricultural Waste on Organic Matter Degradation. International Journal of Current Microbiology and Applied Sciences. 2016; 5(12):23-35.
11. Priyanka A, Nidhi R, Devendra SR, Sonika J. Influence of Chemical fertilizers and Organic fertilizers on pH and available Nitrogen content of Vermicompost with earthworm Eisenia foetida. International Journal of Advanced Research. 2015; 3(7):1030-1036.
12. Reinecke AJ, Viljoen SA, Saayman RJ. The suitability of Eudrilus eugeniae, Perionyx excavatus and Eisenia foetida (Oligochaeta) for vermicomposting in Southern Africa in terms of their temperature requirements. Soil Biology and Biochemistry. 1992; 24:1295-1307.
13. Shoham Y, Lamed R, Bayer EA. The cellulosome concept as an efficient microbial strategy for the degradation of insoluble polysaccharides. Trends in Microbiology. 1999; 7(7):275-281.
14. Subbiah BV, Asija CL. A rapid method for the estimation of available nitrogen in soil. Current Science. 1956; 25:259-260.
15. Suthar S, Watts J, Sandhu M, Rana S, Kanwal A, Gupta D *et al*. Vermicomposting of Kitchen Waste by using Eisenia Foetida (Savigny). Asian Journal of Microbiology, Biotechnology & Environmental Sciences. 2005; 7:541-544.
16. Yasmin S, D'Souza D. Effect of pesticides on the reproductive output of Eisenia fetida. Bulletin of Environmental Contamination and Toxicology. 2007; 79(5):529-532.
17. Yin H, Zhao X, Du Y. Oligochitosan: a plant diseases vaccine-A review. Carbohydrate Polymers. 2010; 82:1-8.