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Microbial Analysis of Compost Using Cowdung as Booster

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Abstract: Microbial analysis of compost using cow dung as booster was investigated. Compost supported high population levels of bacteria with 86% of cultures tested being gram-positive and negative. The microbial identification system based on biochemical analysis identified different groups of bacteria. The microorganisms isolated and identified were *Bacillus pumilus*, *B macereans, B. sphearicus B laterosporus, Micrococcus varians, Proteus mirabilis, Enterobacter aerogenes* for bacteria, while *Aspergillus rapens, A niger, A. flavus, A. fumigatus, Mucor mucedo, Rhizopus stolonifer, Varicosporium* and *Fusarium*, were isolated for fungi. The microbial loads of the compost varies between 1.6 x 10^6 and 1.2 x 10^7 cfu/ml for bacteria, 5.0 x 10^4 and 5.0 x 10^7 cfu/ml.

Key words: Booster, compost, cowdung

Introduction

Composting a natural biological process is the controlled decay of organic matter in a warm moist environment by action of bacteria, fungi and other organisms (Salvator and Sabee, 1995). It requires conditions that are favorable for microbial growth. The process can either be anaerobic or aerobic, but it is much faster and less odourferous if done aerobically.

Composting provides a means of recycling solid wastes and has the potential to manage most of the organic material in the waste stream including restaurant waste, leaves, farm wastes, animal manure, paper products, sewage sludge and domestic wastes. The organic waste materials mainly of animal and plant origin are potential sources of organic matter and plant nutrient (Adeniran *et al*., 2003) and the benefits derived from the utilization of this organic materials ranges from improvement of soil fertility to a reliable means of waste disposal. (Gray and Briddlestone, 1981) report that processing of organic waste by compost will provide an opportunity to reduce bulk and odour while increasing the nutritive values of the materials.

Cow dung manure is a nitrogen rich material and is of economic importance as fertilizer, feed supplement or as energy sources. Cow dung manure has been collected and used to supply nitrogen, potassium, phosphorous and calcium to the soil for plant production (Smith and Wheeler, 1979). Cow dung has a relatively high carbon to the nitrogen ratio.

There are larger organisms present in compost known as the physical decomposers that chew and grind their way through compost heap and are higher up in the food chain (Stainer *et al*., 1998). Most of these groups of decomposers function best at moderate or mesophilic temperature. Examples of the physical decomposers are sow bugs, worms, mites, millipedes, spring tails and flies.

The objective of this study is the determination of microbial load and types of microorganisms responsible for decomposition of compost.

Materials and Methods

The organic waste materials used in the composting process include beans husk, cassava peels, plantain peels, and cow dung. Cow dung was collected inside a sterile polythene bag from the animal farm of the Federal University of Technology, Akure. Yam peels, were collected from major restaurant in the Akure municipality. Cassava peels were collected from a major cassava processing centre in Shagari Village area of Akure. The cassava peels consisted of small chopped cassava tuber and stalk. Beans husk was collected from a bean cake seller and the plantain peels were collected at a domestic waste refuse dump around Akure in Ondo State. All non-compostable materials contained in the wastes were sorted out and not included in the compost preparation.

The waste materials with the exception of the cow dung were reduced to 5mm in size with a shredder. The waste materials were weighted to measure 1kg and each was put into four compost bin.

The waste material in the compost bins were then thoroughly mixed with cow dung in a ratio 1:2:3 (1kg of the cow dung to the first compost bin, 2kg to the second and 3kg to the third compost bin). A control (CCC) was set up in which 1kg of each waste materials were mixed together in a compost bin to form a compost mould without the addition of cow dung.

The windrow compost method was used in which it was not covered and ventilation was not provided through the use of pipes. The aerated bin was constructed using “4 x 4” pallets fastened together to form a box. The compost were watered at the beginning of the composting process and when they were turned.

On the first day of the composting, and throughout the process period, parameters such as temperature humidity, p^H, moisture content, microbial load, and microorganism identification were determined. The temperature of the compost was measured using a mercury thermometer graduated in degree centigrade. The temperature measurement was done daily for about two weeks. Thereafter, the temperatures were done at three days interval until full compost maturity stage.

The P^H of the compost was done at the beginning of compost preparation and subsequently repeated weekly until full maturity stage. Glass electrode p^H meter was used for the determination. Hygrometer graduated in percentage was used for the determination of relative humidity. Moisture contents of the compost was determined by using Mettler LJ 16 moisture analyzer. This was determined weekly until maturity.

The microbial load of the compost was determined at the beginning of the compost preparation and subsequently weekly until full compost maturity stage. The population of microbes mainly bacteria and fungi was determined by using nutrient agar for bacteria enumeration and potato dextrose agar for fungi. Samples were collected from each of the samples respectively using a spatula sterilized with 70% alcohol. The spatula was used to mix the compost mould slightly and to transfer the compost sample into a sterile container and sealed tightly before been taken for microbial analysis.

Moulds on the compost were characterized and identified according to (Barnett and Hunter, 1980). Biochemical tests such as gram stain, spore stain, catalase test, motility, acid fast stain, oxidation-fermentation (OF) test, acid from glucose sugar fermentation, starch hydrolysis, citrate utilization, indole production test, growth on Mac Conkey Agar, growth in air, nitrate reduction, urease production and growth in sodium chloride (NaCl) were carried out on the bacterial isolates using conventional techniques. Chemical analysis of the composts such as ash content, nitrogen, phosphorus carbon, potassium calcium, magnesium sulphur, iron, magnesium, zinc, copper were determined according to (AOAC, 1994) and (Milner and Whiteside, 1984).

Results

Temperature regime of the composts: The different temperature variation during composting is illustrated in Table 1.

The rise and fall in pH in the composts (CC1, CC2 and CC3) were very similar as the results shown in Fig. 1. Showed that initially there was a sharp increase in compost temperature that reached a maximum level (40-45°C) as early as in the first five days of composting. This moderately high temperature was maintained for the first few days of composting and the temperature gradually declined for about 2 weeks and then dropped sharply in the following weeks. Turning of the composts were done after the period of 10-12 days resulting in an increase in temperature which often drops gradually

Table 1: The Temperature Range of Composts

Time (days)	CC1 °C	CC2 °C	CC3 °C	CCC °C	Ambient Temp.
0	40	43	42	42	34
1	41	44	42	43	32
2	42	45	40	43	31
3	42	40	41	44	29
4	42	40	40	45	30
5	40	38	38	44	29
6	39	37	35	44	28
7	37	36	36	42	28
8	37	34	32	41	30
9	38	35	32	40	30
10	37	37	31	40	28
11	36	38	29	39	28
12	34	36	30	38	28
13	34	34	32	37	30
14	34	31	31	38	30
15	34	31	29	36	30
*	33	31	28	34	29
*	32	27	28	35	28
*	29	28	28	35	28
*	28	31	27	35	28
*	31	30	30	34	27
*	31	30	29	33	28
*	30	29	27	32	29
*	28	28	29	32	30
*	29	28	28	30	30
*	28	29	27	31	28
*	28	27	28	29	26
52	-	28	28	31	27
55	-	-	-	30	29

KEYS

CCC: Control compost

CC1: Cow dung compost ratio 1:1

CC2: Cow dung compost ratio 1:2

CC3: Cow dung compost ratio 1:3 * : 3 days interval : Not done

Temp: Temperature

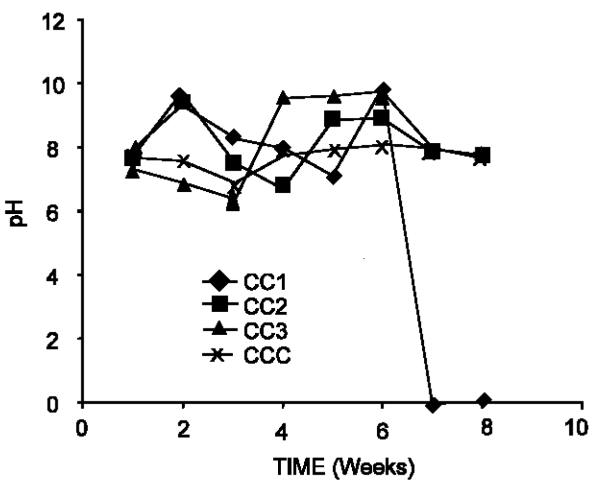

Fig. 1: Effect of pH on composting on time (days)

Table 2: Chemical Composition of the Matured Composts

Compost	PPM Composition							% Composition	
	Ca	Mg	K	P	Fe	Cu	Cr	N	C
CCC	9.00	1.38	658.90	790.99	45.84	0.12	3.82	0.83	93.04
CC1	1,300	600	1013.22	509.80	111.52	18.64	11.15	2.21	55.46
CC2	1,400	800	137.87	464.27	86.56	22.66	12.59	1.63	53.84
CC3	2,000	800	165.01	666.57	92.87	18.87	14.34	1.89	70.14

within a short time. Consequently, every other turning caused a rise in temperature for a short period of time.

Composts reached maturity within 40-45 days at this point the temperature remained almost constant (between 28 and 30°C). The CC1 compost matured earlier than those of the other composts (CC2 and CC3) while the control (CCC) composts took a longer time to mature because of its slow rate of decomposition. At the later stages of compositing, the composts temperature remain higher (28-31°C) than the ambient temperature (27-29°C).

The pH values for the composting ratios varies at the beginning of composting and ramped from 7.3 to 7.7 as the composting proceeded.

The p^H decreases slightly and reached minimum volume in the first 3 weeks of composting. After this initial decline, the composts showed a gradual rise in p^H to a minimum level of about 9.58 for CC1, 8.82 for CC2, 9.63 for CC3 and 8.0 for CCC. At the later stages of composting (few weeks to maturity), a decline in the p^H values was observed for compost.

The moisture content values of the composts were considerably high in the first 3 weeks of composting and after which it increased considerably in the later weeks.

The concentrations of plant nutrients in the four composts as influenced by the ratio of the cow dung proportion are presented in.

The nutrients concentration varied slightly depending on the ratio of the composts. Total nitrogen, organic carbon and some micronutrients in the composts were significantly lower in CCC than in other compost.

The population of microorganisms contributing to the composting process are numerous and the population varies in the composting ratios as shown in Table 3.

Discussion

Composting is a dynamic process which will occur quickly or slowly depending on the process used and still with which it is executed. The success with which organic substances are composted depends on the organic material and the decomposer organisms involved. The organic materials used in these experiments varied in nutrients composition and after composting give different concentrations of plant nutrients. The lower C: N ratio in cow dung manure (high nitrogen content) is an indication that the former could be good source of protein for the microbes involved in the decomposition of organic materials.

The rate of decomposition of the composts was observed and the results from the study showed that the control (CCC) took longer time to decompose or mature than the other composts, potentially due to non addition of the dung which has a high mineral and nutrient content favourable for microbial growth and activities. The CC3 matured earlier than other composts due to the high microbial activity in the CC3 compost. The CC3 was able to retain its moderately high temperature for a longer time compared to the other composts as a result of the large quantity of the composting material in CC3. The temperature regime in the various composts indicated that the organic materials passed through almost similar degradation processes. During composting, the temperature pattern of the compost followed similar results obtained in many other composting systems (John *et al.*, 1995). The ambient temperature played a part in the resulting temperature pattern of the composts.

The general rise in temperature of the compost in the early stage of composting was caused by rapid mineralization of organic carbon and nitrogen in the presence of adequate aeration and moisture as required by microbes responsible for the breakdown of organic compounds. This probably would have generated reactions whereby CO_2 and heat were released into the compost system (Foth, 1980). A drop in temperature in the compost pile before material is stabilized can mean that the pile is becoming anaerobic and should be aerated. The temperature curve for the composts varies somewhat with the size of pile, the ambient temperature, the moisture content, the degree of aeration and the character of composting material. The mesophilic temperature got from the composts is responsible for the longer times required for decomposition.

The results from this study indicated that processes such as thorough mixing of the material, turning and watering of the composts enhanced the decomposition process. The turning ensures an adequate supply of oxygen to the microbes and at the later stages the turning process fails to reheat the composting pile indicating that the compost materials is biologically stable. The moisture content of the compost was considerably high and the moisture content values were maintained by preventing the excess watering of the composts. The moisture content values of the composts increased gradually as the composts matured indicating

Table 3: Composting is a Dynamic Process

Weeks	CC1		CC2		CC3		CCC	
	BAC	Fungi	BAC	Fungi	BAC	Fungi	BAC	Fungi
1	36 x 10^6	32 x 10^6	40 x 10^6	50 x 10^6	46 x 10^6	13 x 10^6	23 x 10^6	11 x 10^6
	128 x 10^5	98 x 10^5	47 x 10^5	16 x 10^5	77 x 10^5	23 x 10^5	68 x 10^5	42 x 10^5
2	24 x 10^6	16 x 10^6	17 x 10^6	4 x 10^6	10 x 10^6	2 x 10^6	19 x 10^6	7 x 10^6
	94 x 10^5	9 x 10^5	98 x 10^5	5 x 10^5	47 x 10^5	12 x 10^5	47 x 10^5	23 x 10^5
3	24 x 10^6	16 x 10^6	17 x 10^6	4 x 10^6	10 x 10^6	2 x 10^6	19 x 10^6	7 x 10^6
	76 x 10^5	5 x 10^5	58 x 10^5	7 x 10^5	33 x 10^5	42 x 10^5	28 x 10^5	19 x 10^5
4	2 x 10^6	1 x 10^6	6 x 10^6	2 x 10^6	18 x 10^6	4 x 10^6	11 x 10^6	3 x 10^6
	12 x 10^5	25 x 10^5	15 x 10^5	4 x 10^5	54 x 10^5	12 x 10^5	23 x 10^5	22 x 10^5
5	23 x 10^6	2 x 10^6	26 x 10^6	7 x 10^6	16 x 10^6	7 x 10^6	8 x 10^6	5 x 10^6
	70 x 10^5	15 x 10^5	37 x 10^5	20 x 10^5	60 x 10^5	15 x 10^5	33 x 10^5	17 x 10^5
6	14 x 10^6	3 x 10^6	21 x 10^6	7 x 10^6	21 x 10^6	3 x 10^6	7 x 10^6	3 x 10^6
	32 x 10^5	9 x 10^5	54 x 10^5	12 x 10^5	64 x 10^5	12 x 10^5	27 x 10^5	11 x 10^5
7	-	-	14 x 10^6	3 x 10^6	17 x 10^6	2 x 10^6	4 x 10^6	2 x 10^6
	-	-	58 x 10^5	19 x 10^5	58 x 10^5	14 x 10^5	18 x 10^5	13 x 10^5
8	-	-	10 x 10^6	1 x 10^6	15 x 10^6	2 x 10^6	5 x 10^6	3 x 10^6
	-	-	48 x 10^5	4 x 10^5	23 x 10^5	17 x 10^5	186 x 10^5	10 x 10^5

KEYS: CCC: Control compost CC1: Cow dung compost ratio 1:1
CC2: Cow dung compost ratio 1:2 CC3: Cow dung compost 1:3 -: Not done. BAC: Bacteria

that the composting microorganism utilizes the moisture and that they thrive in the moist conditions. Studies show an important and significant correlation between the moisture content and the temperature distribution within the compost pile (Foth, 1980). The increased surface area of the organic materials by shredding makes the micro organisms to digest more material, multiply more rapidly and generate more heat which invariably speeds up the composting process.

The microbial load of the compost was found to be on the increase any time the compost was subjected to turning, suggesting that the turning procedure increased oxygen level in the compost system. The growth of microorganisms in the compost pile as seen in Table 3 was on the increase in the first 3 weeks due to synthesis and utilization of the various nutrient present in the compost by microorganisms. (Hargerty *et al.*, 1999) reported that there was maximum increase in microbial population of composition of the compost and the favorable environmental conditions of the compost. This phase of growth or rapid activities by the microorganism can be described as the log phase. Subsequently at the later week of composting, there was a gradual decline in the microbial population as seen in Table 3. The remaining microorganisms were those that were able to survive the mesophilic range of temperature present in the compost system, hence, the rate of increase in population was dependent on the supply of nutrients in the compost and other environmental factors of the compost such as temperature, aeration and moisture content.

Reasonable amount of microorganisms were still present in the compost at maturity. Fungi population in all the compost was greatly reduced when the compost system was nearing maturity. *Aspergillus niger* was the predominant fungi isolated from the compost at the latter weeks. The presence of *Aspergillus niger* could have been aided by its ability to adapt to the moderately high temperature of the compost (25-30^{o}C) as reported by (Gray and Briddlestone, 1981). The bacteria identified were *Micrococcus* varians, *Micrococcus luteus, Bacillus pumilus, Bacillus macereans, Bacillus spaericus*, *Bacillus* laterosporus, *Psudomonas aeroginnosa, Enterrobacter aerogenes, Proteus mirabilis*.

The fungi identified were *Aspergillus niger, Aspergillus flavus*, *Aspergillus rapens, Rhizopus stolonifer, Mucor mucedo, Fusarium*, *Aspergillus fumigatus, Varicosporium.*

The groups of microorganism isolated from the compost were able to survive under the mesophilic range of temperatures of the compost, the neutral p^H of the composts and the moderately high moisture content of the compost. (Blanc *et al.*, 1997) isolated *Bacillus* species from hot compost and also reported that *Bacillus* species are among the groups of the bacterial isolated from compost. *Bacillus* species occurs in soil water, air and on vegetation. They are able to survive in the compost pile due to their adaptability to mesophilic temperature in the compost. Also, the *Pseudomonas* species is very nutritionally versatile and capable of degrading many natural and synthetic organic compounds (Stainer *et al.*, 1998). They are aerobic and contribute to the decomposition and nutrient release process by attacking a wide variety of organic substrate including humid acids and synthetic pesticides (Murray *et al.*, 1990). *Pseudomonas* species are able to survive in the first two weeks of composting and after which they were eliminated probably due to unfavorable environmental conditions at the early period of composting.

Enterobacter aerogenes is common in soil, water and mainly act as saprophytes (Murray *et al.*, 1990). *Proteus* *mirabilis* are soil inhabitants found particularly abundant in decomposing animal materials and occurs in the environment as saprophytes (Stainer *et al.*, 1998). *Proteus* can be readily recovered from soil, sewage, garden vegetation and organic materials (Cruickshark *et al.*, 1973).

The different fungi isolated from the different composts could be classified as saprophytes. They obtain energy by breaking down the final stages of the compost pile when the compost has been changed to a more easily digested form. According to (Hargerty *et al.*, 1999). *Aspergillus* species are among the predominant fungi in compost as they classified them as thermophilic fungi in composting which degrade or break down the organic waste.

Aspergillus fumigatus is a common fungus of compost. It is strongly cellulolytic and can enter the lungs as inhaled spores causing aspergillosis. It has the ability to grow readily at 37°C.

Apergillus niger occurs everywhere including the air and soil. They were found predominantly in the composts virtually on a weekly basis during isolation from the compost. *Mucor* species is ubiquitous in the environment and is found on organic material acting as saprophytes and could cause mycosis in man. (Rhode and Hartman, 2002).

Rhizopus stolonifer is a ubiquitous organism in the environment and are encountered as contaminate. With a temperature rage of 25-45°C obtained from the compost, this fungus is a typical early colonizer of the compost exploiting simple sugars and amino acids that are present initially in the organic material. During the latter stages of composting they are inactivated and did not recolonize afterwards.

The effective action of the different microorganisms resulting in the decomposition of organic material resulted in the overall reduction in the size of the compositing material to give a deep brown chocolate compost colour.

In conclusion, the production of high quality dark brown compost can be enhanced by microorganisms, temperature, aeration, moisture content and chemical composition of the organic waste materials. The cow dung facilitated the early maturity of the compost and also contributed to the nutritional content of the composts. The different organic materials used in the composting process were totally utilized and converted to nutrient rich compost, hence making composting an excellent waste recycling process. In general, the production and use of compost helps to increase soil nutrients and to improve some properties of the soil such as pH, texture, soil aggregation and chemical composition of the soil.

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