ISSN: 2319-8753

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 3, Issue 6, June 2014

Seasonal Variations of Microbial Populations during Composting Processes of Municipal Solid Wastes

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ABSTRACT: This study investigates the prevailing seasonal changes of physico-chemical and microbial community for mesophilic bacteria and fungi at different degradations stages of municipal solid wastes. The samples were collected from Excel plant (Vidyaranyapuram, Mysore) in different depths of pile during summer, rainy and autumn seasons in the year of 2011 to 2012 at once in 10 days intervals up to 60 days. Temperature and pH were measured by using standard method. The microbial analysis was done by serial dilution method and bacterial growth Nutrient agar (NA) and Czapek Dox Agar (CDA) for fungi enumeration. The pure cultures of the bacterial isolates were subjected to various morphological and biochemical characterization tests like, Catalase Test (CAT), Oxidase Test (OXT), Indole Test (INT), Methyl Red Test (MRT), Voges-Proskauer Test (VPT), Citrate Utilization Test (CUT), Urease Test (URT), Nitrate Reduction Test (NRT), Hydrogen Sulphide Production (H_2S), Starch Hydrolysis Test (SHT) and Gelatine Hydrolysis Test (GHT) to determine the identity of the bacteria isolates. The results reveal that, the temperature of the windrows in all seasons reached maximum after 4 weeks of composting and then decreased by the end of the composting period (60 days). Marked changes in pH values of the composts in all seasons during degradation stages were found, but final stages shows the pH was at neutral except rainy season (60 days old compost sample with 8.7). The microbial populations were significant increase during initial stages of composting process and final stages pathogenic microbes was reduced, for all the three seasons. The *Bacillus* Sp., *Pseudomonas* and *Asprigillus* Sp., was dominate species during composting process. From the present investigation, it can be concluded that, the summer and autumn seasons microbial activities faster because the favourable environmental conditions for supporting the proper wastes degradation, therefore, these two seasons for obtained better quality of compost than rainy season.

KEYWORDS: Municipal Solid Waste, Physico-chemical characteristics, Biochemical characterization test, Microbial communities

I. INTRODUCTION

Composting is a natural process of decay. Basically aerobic decomposition carried out under controlled conditions of ventilation, temperature, moisture and micro-organisms in the waste themselves that convert waste into humus-like material by acting on the organic portion of the solid waste (Sathishkumar, *et.al.,* 2002). The important mention is that, a large variety of mesophilic, thermo tolerant and thermophilic aerobic micro-organisms, including bacteria, actinomycetes, yeasts and various other fungi have been extensively reported in compost and other self heating organic materials (Amner *et.al.,* 1988; Faure and Deschamps, 1991; Finstein and Morris, 1975; Strom, 1985; Beffa *et.al.,* 1996). Many factors determine the microbial community during composting. Under aerobic conditions, temperature and moisture content are the major factor that determines the types of micro-organisms, species diversity and rate of metabolic activities. During composting process, the solid waste was auto-sterilization induced to help in the destruction of pathogens (Golueke, 1977). On the other hand, the composting process can, if not properly managed induced the increasing and spreading of potential pathogenic or allergic thermo-tolerent or thermophilic fungi and bacteria (Beffa, *et.al.,* 1996). Among the bacteria, Salmonella, Shigella, Escherichia coli, Enterobacter, Streptococci and klebisella can emerge and cause infections for compost handlers and agricultural users (Strauch, 1996).

ISSN: 2319-8753

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Composting satisfies the health and aesthetic aspects of waste disposal by destroying almost all pathogens (Walker, 1973). In addition, the product was agriculturally and horticultural used to improve soil fertility and plant nutrition. The aim of this paper is to observe bacterial population and fungal count variations according to the seasons, during organic matter transformation in the compost material at successive maturity stages

II. MATERIALS AND METHODS

Mysore has a warm & a cool climate throughout the year and it is one of the famous tourist places in Karnataka State. The latitude and longitude of the city is 12^0 18' N and 76^0 39'. The amount of waste generated in Mysore city is average of about 300.9 tons per day (Shyamala and Belagali, 2012).The Municipal Solid Waste (MSW) aerobic compost treatment plant situated in Vidyaranyapuram Mysore city and this treatment was initiated by private origination. The samples were drawn during three seasons, summer (April-May), rainy (June-July) and autumn season (November to December) in the year 2011-12, at different stages of the composting process like-10, 20, 30, 40, 50 and 60^{th} days.

Bacterial and fungal enumerations methods:
The samples were collected in a separate sterilised polythene bags for biological characteristics. The compost sample (1 g) was diluted with 9 ml of saline solution (0.9 g NaCl/100 ml of sterilized distilled water) (1/9 v/v) to get pH 7.6. Decimal serials dilutions (10^{-1} to 10^{-10}) were made and inoculated aseptically in Petri dishes (1000 µL) with different culture media: Nutrient agar (NA) and Czapek Dox Agar (CDA) in order to facilitate the growth of bacteria and fungi, respectively. Petri dishes were incubated at 37^0 C for 24h (NA) and 30^0 C for 72h (CDA). After incubation isolated colonies of bacteria and fungi were selected. The evaluation of cellular concentration in a compost sample was determined by plate count by serials dilutions method, according a to equation 1:

CFU/g = Colonies Numbers X dilution /100 ----------eq.1

The number of mesophilic bacteria determined by dilution plate count technique as described by Hernesmaa *et al.,* (2008).

Phenotypic characterization:
The phenotypic characterization of all isolates studied were performed and compared to phenotypic data of known organisms described in the Bergey's Manual of Systematic Bacteriology (Buchanan and Gibbons 1986). The phenotypic features characterized are as follows: Cultural and Morphological characteristics: The colony morphology, cell morphology and the motility of bacterial isolates from fresh cultures were evaluated.

Isolation and purification of bacterial isolates: Bacterial colonies were isolated with differential colony morphology study. After isolation, the isolates were purified and characterised by biochemical tests.

Biochemical tests:
A number of biochemical tests and gram staining were performed for the identification of bacterial isolates with the help of Bergey's Manual (Buchanan and Gibbons 1986). The principal tests used for this purpose are Catalase Test (CAT), Oxidase Test (OXT), Indole Test(INT), Methyl Red Test (MRT), Voges-Proskauer Test (VPT), Citrate Utilization Test (CUT), Urease Test (UT), Nitrate Reduction Test (NRT), Hydrogen Sulphide Production (H_2S), Starch Hydrolysis Test (SHT) and Gelatine Hydrolysis Test (GHT). Catalase test was performed by adding a small amount of bacterial isolate into freshly prepared 3 % hydrogen peroxide, and the bubbles of oxygen if appeared, the isolate was considered as positive for catalase test.
Oxidase test was used to assess the bacteria which produce the enzyme Cytochrome Oxidase. Trypticase soy agar was inoculated and incubated the plates in an inverted position for 24 to 48 hours at 37^0 C. After incubation, a few drops of 1 % tetramethyl-p-phenylenediamine dihydrochloride were added. A positive result was the development of purple color. No color change indicated a negative result. For Indole test was performed by culturing the microorganisms in peptone water medium containing tryptophan in a screw capped tube, incubated for 24 h at 37^0 C and then Kovac's reagent (0.5 ml) was added where the positive results was indicated by the formation of pink red layer on the broth within seconds of adding Kovac's reagent. Methyl red test was performed by inoculation of the glucose phosphate

ISSN: 2319-8753

International Journal of Innovative Research in Science, Engineering and Technology

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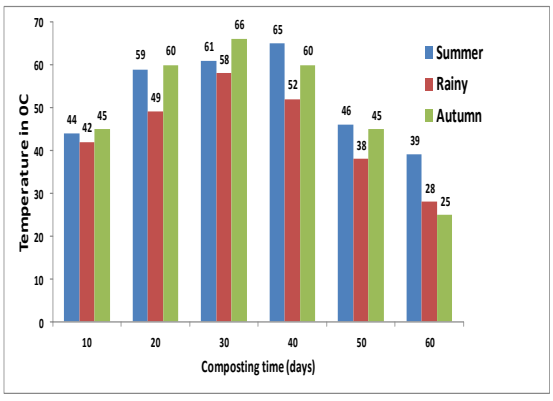
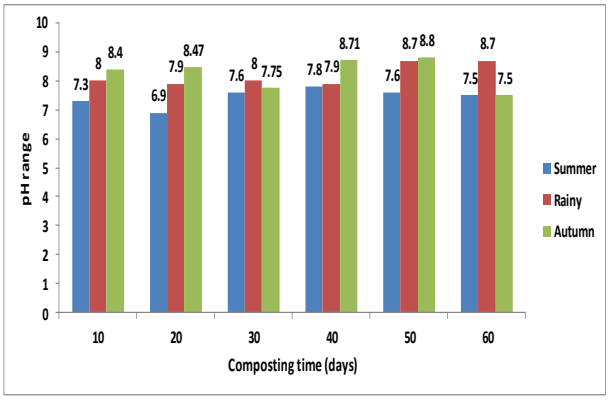
peptone water in a screw capped tube, incubation for 24 to 48 h and then addition of 5 drops of methyl red, where the change in color of the medium to cherry red was considered as positive. Voges-Proskauer test was performed by inoculating glucose phosphate peptone water with the microbial isolates in a screw capped tube, incubating for 24-48 h, then adding 0.6 ml of alpha-naphthol solution and 0.2 ml of Potassium Hydroxide solution (Barritti's reagent). The tubes were then allowed to study for 5-10 min after shaking well. The red color formation was taken as the positive result. For the Citrate utilization test, the Simons citrate agar were inoculated and incubated at 37^0 C for 24-48 h. The positive slants were noted to change color from green to blue. The Urease test, urea broth was inoculated and incubated at 37^0 C for 24 to 48 h. The change of color of the broth from yellow-orange to bright pink was considered as positive. The Nitrate reduction test, nitrate broth was inoculated and incubated at 37^0 C for 24-48 h. After incubation, 5 drops of Sulfanilic acid and 5 drops of N, N -dimethyl-1-naphthylamine were added. The change of color of broth to deep red within 5 min means show that, the bacteria had produced nitrate reductase. If color did not change, the result was indecisive. Small amount of Zinc was added to the broth. If the solution remains colourless, then both nitrate reductase and nitrite reductase are present. If the solution turns red, nitrate reductase was not present. The Hydrogen sulphide production test, was used to differentiate species of the family Enterobacteriaceae. This test was used to determine the ability of an organism to reduce sulphur into H_2S. Sulfide-Indole-Motility (SIM) media was used for the H_2S production test. Sulfide-Indole-Motility media contains the sulfur containing amino acid, sodium thiosulfate, cysteine, and ferrous sulfate. The sulfide-indole-motility media was inoculated with bacterial cultures by stabbing SIM media with inoculating needle. The tubes were then incubated at 35^0 C for 24 h. After incubation, a positive result was indicated by a black precipitate formed because of the reaction of H_2S with the iron or ferrous sulfate; while the negative result was indicated by without black precipitate. The starch hydrolysis test was used to differentiate bacteria based on their ability to hydrolyze starch with the enzyme alpha-amylase or oligo-l, 6-glucosidase. Starch agar was inoculated and incubated the plates in an Inverted position for 48 hours at 35°C and then iodine 0.5 ml of reagent was used to detect the presence or absence of starch in the vicinity around the bacterial growth. A positive result was the development of a blue or dark brown color; therefore, any microbial starch hydrolysis will be revealed as a clear zone surrounding the growth. The gelatine hydrolysis test was used to determine the ability of a microbe to produce gelatinases. Staphylococcus aureus which is gelatinase-positive can be differentiated from S. epidermidis. Serratia and Proteus species are positive members of Enterobacteriaceae while most others in the family are negative. Bacillus anthracis, B. cereus and several other members of the genus are gelatinase-positive. The presence of gelatinases can be detected using nutrient gelatine and the tubes were then incubated at 25^0C for up to one week. After incubation, a positive result was indicated a solid form gelatine is to liquid form this is because of the gelatinases-positive organism, secreted gelatinase and negative results shows that, the solid form of gelatine media as its.

Identification of Fungal Morphology by Staining: Each fungal colony was picked up at the 7^{th} day of culture incubation, placed onto a glass slide, stained with a few drops of cotton blue dye (6 µg/mL) by spreading the sporulated fungi with the help of a sterile needle. The stained material was covered with glass cover slip and visualized under the compound microscope by the LYNX Trinocular stereo zoom microscope model LM-52-3622 and study the morphological characteristics of the fungi.



Graph 1: Seasonal variations of Temperature



Graph 2: Seasonal variations of pH

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Table 1: Bacterial density from the different degradation stages of compost samples

Season	Sample	CFU/g	Season	Sample	CFU/g	Season	Sample	CFU/g
Summer	10 days	$1.8X10^9$	Rainy	10 days	$1.9X10^9$	Autumn	10 days	$1.8X10^9$
	20 days	$4.0X10^9$		20 days	$3.9X10^9$		20 days	$4.0X10^9$
	30 days	$5.0X10^9$		30 days	$6.9X10^9$		30 days	$6.0X10^9$
	40 days	$3.0X10^9$		40 days	$7.2X10^9$		40 days	$6.0X10^9$
	50 days	$2.2X10^9$		50 days	$3.2X10^9$		50 days	$4.2X10^9$
	60 days	$1.0X10^9$		60 days	$1.8X10^9$		60 days	$1.0X10^9$

Table 2: Seasonal changes in selected bacterial colonies during the composting of municipal solid wastes at Vidyaranyapuram compost treatment plant

Composting time (days) for bacteria identified																		
	Summer season						Rainy season						Autumn season					
Sampling days	10	20	30	40	50	60	10	20	30	40	50	60	10	20	30	40	50	60
Klebsiella sp.,	+	+	+	+	-	-	+	+	+	+	-	+	+	+	+	+	+	-
Pseudomonas sp.,	+	-	-	+	+	+	+	-	-	+	+	-	-	-	-	+	-	-
Staphylococcus aureus	+	+	-	-	-	-	+	-	-	+	-	+	-	-	-	+	-	-
Enterobacter aerogenes,	+	+	+	+	-	-	+	+	+	+	+	-	+	+	-	+	-	-
Salmonella,	+	+	-	-	-	-	+	+	-	-	-	-	+	+	-	-	-	-
Bacillus sp.,	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Escherichia coli,	+	+	-	+	-	-	+	+	-	+	-	-	+	+	+	+	-	-
Flavobacterium sp.,	-	+	+	-	+	-	-	+	+	+	-	-	+	-	-	-	-	-
Staphylococcus xylosus,	-	-	+	+	-	-	-	+	+	+	-	-	-	+	+	+	-	-

Table 3: Morphological characteristics of selected isolates

Bacterial morphology	Form	Surface	Colour	Margin	Elevation	Opacity
Klebsiella sp.,	Irregular	Glistering	Cream	Entire	Raised	Opaque
Pseudomonas sp.,	filamentous	Glistering	White	Lobate	Umbonate	Rough
Staphylococcus aureus	Circular	Smooth	Yellowish	Entire	Raised	Opaque
Enterobacter aerogenes,	Circular	Shiny	White	Entire	Convex	Moist
Salmonella,	Circular	Smooth	colourless	Lobate	Slightly raised	Opaque
Bacillus sp.,	Circular	Smooth	Cream	Undulate	Raised	Opaque
Escherichia coli,	Circular	Smooth	Whitish	Entire	Convex	Translucent
Flavobacterium sp.,	Circular	Smooth	Yellowish	Entire	Convex	Translucent
Staphylococcus xylosus	Circular	Smooth	White	Irregular	Raised	Opaque

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Table 4: Biochemical observations of selected isolates

Microbe identified	CAT	OXT	CUT	H_2S	MRT	VPT	NUT	INT	URT	SHT	GHT	GRAM STAIN
Klebsiella sp.,	+	-	+	-	-	+	+	+	+	ND	ND	-ve
Pseudomonas sp.,	+	+	+	-	+	-	+	-	-	-	-	-ve
Staphylococcus aureus	+	-	-	-	+	-	+	-	+	ND	ND	+ve
Enterobacter aerogenes,	+	-	+	-	-	+	+	-	-	-	-	-ve
Salmonella,	+	-	+	-	+	-	+	-	-		-	-ve
Bacillus sp.,	+	-	+	-	+	-	-	-	-	-	+	+ve
Escherichia coli,	+	-	-	-	+	-	+	+	-	-	-	-ve
Flavobacterium sp.,	+	+	-	-	-	-	+	-	ND	+	+	-ve
Staphylococcus xylosus	+	+	-	-	-	-	+	-	+	ND	ND	+ve

Note: Catalase Test (CAT), Oxidase Test (OXT), Indole Test (INT), Methyl Red Test (MRT), Voges-Proskauer Test (VPT), Citrate Utilization Test (CUT), Urease Test (URT), Nitrate Reduction Test (NRT), Hydrogen Sulphide Production (H_2S), Starch Hydrolysis Test (SHT), Gelatine Hydrolysis Test (GHT).

Table 5: Fungal density from the different degradation stages of compost samples

Season	Sample	CFU/g	Season	Sample	CFU/g	Season	Sample	CFU/g
Summer	10 days	$0.6X10^9$	Rainy	10 days	$0.5X10^9$	Autumn	10 days	$0.3X10^9$
	20 days	$2.4X10^9$		20 days	$3.0X10^9$		20 days	$2.0X10^9$
	30 days	$3.4X10^9$		30 days	$4.2X10^9$		30 days	$3.0X10^9$
	40 days	$4.0X10^9$		40 days	$5.1X10^9$		40 days	$3.0X10^9$
	50 days	$1.9X10^9$		50 days	$2.5X10^9$		50 days	$2.2X10^9$
	60 days	$0.1X10^9$		60 days	$2.0X10^9$		60 days	$0.2X10^9$

Table 6: Seasonal changes in selected fungal colonies during the composting of municipal solid wastes at Vidyaranyapuram compost treatment plant

	Summer season						Rainy season						Autumn season					
Fungi identified	10	20	30	40	50	60	10	20	30	40	50	60	10	20	30	40	50	60
Aspergillus niger,	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	-
Aspergillus flavus	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	-

ISSN: 2319-8753

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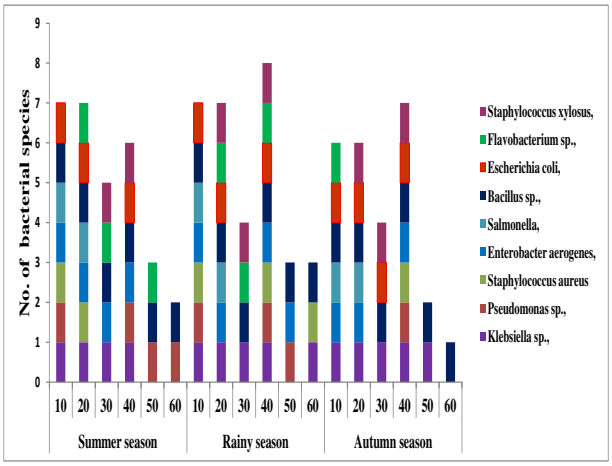
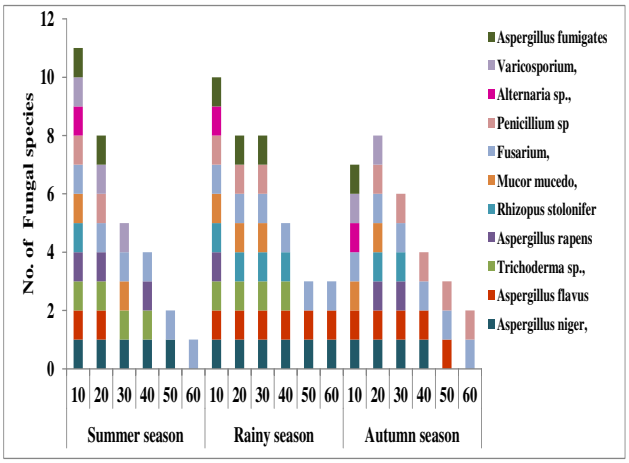
Trichoderma sp.,	+	+	+	+	-	-	+	+	+	+	-	-	-	-	-	-	-	-
Aspergillus rapens	+	+	-	+	-	-	+	-	-	-	-	-	-	+	+	-	-	-
Rhizopus stolonifer	+	-	-	-	-	-	+	+	+	+	-	-	-	+	+	-	-	-
Mucor mucedo,	+	-	+	-	-	-	+	+	+	-	-	-	+	+	-	-	-	-
Fusarium,	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Penicillium sp	+	+	-	-	-	-	+	+	+	-	-	-	-	+	+	+	+	+
Alternaria sp.,	+	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-
Varicosporium,	+	+	+	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-
Aspergillus fumigates	+	+	-	-	-	-	+	+	+	-	-	-	+	-	-	-	-	-



Graph 3: Number of bacterial species present in different composting days



Graph 4: Number of fungal colonies present in different composting days

Physico-chemical characteristics study of compost:

Temperature is one of the most important parameters of compost quality and reflects the microbial activity in composting process (Mustin, 1987). In biological terms, the operating temperature ranges are > 55°C to maximise sanitation, 45-55°C to maximise the biodegradation rate, and 35-40°C to maximise microbial diversity (Stentiford, 1996). In the present investigation, the temperature in summer ranged from 39^0 C to 65^0 C, during rainy 28^0 C to 58^0 C

ISSN: 2319-8753

International Journal of Innovative Research in Science, Engineering and Technology

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and during autumn season, 25^0 C to 66^0 C as given in the graph 1. The temperature was gradually increasing upto 40 days during summer and autumn season and in rainy season, the temperature was lower in composting process. The pattern of pH during the composting process in all seasons was typical for composting processes as described by several authors (Chang & Hundson 1967; Gary 1985; Inbar *et.al*., 1993). The average changes in pH values of compost samples are shown in graph 2. The pH values ranged from 6.9 to 7.8, 7.9 to 8.7 and 7.5 to 8.8 during summer, rainy and autumn seasons respectively and pH got gradually decreased upto 20 days during summer and autumn seasons, which can be attributed to the production of CO_2 from organic acids and loss of nitrogen (Lugtenberg, 2009) and ammonia volatilization could be one of the most important reasons for pH drop (Kim *et al*., 2008 and Elango *et al*., 2009). In rainy season, the pH values showed the fluctuation during composting process and final compost sample was found to be basic with 8.73, because of addition of more nitrogen rich solid waste and yard trimming feedstock (Fauci, *et.al.,* 1999).

Bacteriological study on compost:

Monitoring of the microbial succession is important in the effective management of the composting process as microorganisms play key roles in the process and the appearance of some microorganisms reflects the quality and maturing of compost (Ryckeboer *et. al.,* 2003). Seasonal changes in the number of bacteria and fungi during the composting of municipal solid wastes at Vidyaranyapuram (Mysore City) compost plant are illustrated Tables 1, 2, 3 and 4. In these experiments, total aerobic bacteria per gram compost ranged from 1.0 X 10^9 to 5.0 X 10^9 CFU in summer, 1.8 X 10^9 to 7.2 X 10^9 CFU rainy and 1.0 X 10^9 to 6.0 X 10^9 in CFU autumn season (Table 1).

The decrease was higher in case of summer season than other two seasons which could be attributed to the decrease in moisture content in summer season, where it was unsuitable to mesophilic microbial growth. In case of rainy season, for the final stage compost sample, few more bacteria will be present which may have adverse environmental conditions (due to high moisture content). Autumn seasons are considered to be suitable but not the optimum for composting. The nearest conditions to the optimum were found in autumn season.

The Morphological characteristics of each isolates obtained from the compost samples are given Table 3. The Biochemical characteristics of the isolates obtained from these compost samples is shown in Table 4. The present study most of the isolates bacteria are gram negative and *Bacillus sp., Klebsiella sp., Pseudomonas sp., and Escherichia coli* species were dominant during the entire process of composting. These observations are in conformity with the results obtained by researchers (Fang and Wong, 2000; Gestel *et.al.,* 2003; Pedro *et.al.,* 2003). The *Pseudomonas* species is nutritionally versatile and capable of degrading many natural and synthetic organic compounds (Stainer *et.al*., 1998). The *Bacillus* species from hot compost occurs in soil water, air and on vegetation and they are able to survive in the compost pile due to their adaptability to mesophilic temperature in the compost (Blanc *et.al*., 1997). The decrease of bacterial diversity in the composting mass could be due to the high temperature (Fang and Wong 2000) and the bacterial viable counts were more for 10, 20, 30, 40 days compost samples compared to 50 and 60 days old compost samples.

Fungal study on compost:

As shown in Table 5, the total viable fungi per gram of compost ranged from 0.1 X 10^9 to 4.0 X 10^9 CFU in summer, 0.5 X 10^9 to 5.1 X 10^9 CFU in rainy, 0.3 X 10^9 to 3.0 X 10^9 CFU in autumn seasons and number of mesophilic fungi disappeared first 2 weeks, on the other hand, the thermophilic fungi increased and reached the maximum number after 4 weeks of composting and then gradually decreased (Table 6). Most fungi are eliminated at the temperatures above 50^0 C, only a few were isolated from compost which can grow up to 62^0 C. Their survival was due to their thermotolerance property. These observations were in conformity with those obtained by other researchers according to Hegarty, *et al*., (1999), *Aspergillus* species was among the predominant thermophilic fungi in compost and break down the organic waste.

The common fungi were identified in all the degradation stages like, Aspergillus niger, Aspergillus flavus, Trichoderma sp., Aspergillus rapens, Rhizopus stolonifer, Mucor mucedo, Fusarium, Aspergillus fumigatus, Varicosporium, Alternaria sp., and Penicillium sp. Aspergillus, Fusarium and Penicillium species were dominant during the entire

ISSN: 2319-8753

International Journal of Innovative Research in Science, Engineering and Technology

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process of composting. The presence of Aspergillus niger could have been aided by its ability to adapt to the moderately high temperature of the compost.

However during rainy season, at the final stage of compost sample, the fungal colonies were more compared to summer and autumn seasons, due to adverse environmental conditions. From the results obtained, it can be seen that, the most common micro-organisms in the composting process are bacteria (mesophilic and thermophilic). The mesophilic fungi had a short time span in the composting process. Bacteria flourished because of their ability to grow rapidly on soluble proteins and other readily available substrates and because they are more tolerant of high temperatures. Generally mesosphilic micro-organisms are responsible for the initial decomposition of organic materials and the generation of heat responsible for the increase in compost temperature (Nakasaki *et.al.* 1985c; Fogarty and Tuovinen 1991) and (Hargerty *et. al*., 1999) reported that, there was maximum increase in microbial population of composition of the compost under favourable environmental conditions. On the other hand Ryckeboer *et. al.,* 2003 reported that, microbial population fluctuates mainly by physical parameters (temperature and pH) and climatic condition of the city.

IV. CONCLUSION

From this study, it can be concluded that, physico-chemical & microbiological characteristics of Municipal Solid waste compost of Mysore city, Karnataka, India reveals reduction in microbial counts as well as microbial succession without a definite pattern and lower microbial counts at the end of the composting period and at the same time adjustment of composting conditions such as pile size, aeration, climatic conditions, temperature and moisture content. This would allow the microbial populations and their enzymatic activities to increase and therefore the increase of organic matter decomposition as a reduction in carbon and nitrogen ratio and then the composting time can be reduced. The better quality of compost obtained summer and autumn seasons than rainy season.

V. ACKNOWLEDGMENT

One of the authors Mrs. Shyamala D C, gratefully acknowledges to Department of Science and Technology (DST), Government of India, New Delhi for INSPIRE Fellowship to carryout the research work.

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ISSN: 2319-8753

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

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