



Microbial Biodiversity of Municipal Solid Waste of Ahmedabad

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Abstract

Ahmedabad, Gujarat State is the 7th largest metropolis of India having a population of almost 60 lakhs and spread over an area of 466 sq km and generating 3500 metric tons of solid waste on daily basis. This metropolitan city has two fieldland sites, at Pirana it has been operated since 1980 and another at Gyaspur operated since 2009. The management and right direction disposal of the accumulated domestic solid waste is a crucial challenging task for the municipal and state government authorities. The attempts were carried out to determine the physicochemical properties of the solid waste to justify it as an ideal matrix for composting process. The qualitative and quantitative microbial analysis were carried out for selected samples for the primary screening of potent fungal strains with wide spectrum of nutritional catabolic profile which could be exploited during secondary screening process for the development of ecofriendly, sustainable, efficient composting process.

Keywords: Biodiversity, Composting, Fieldland, Microbial Community, Screening, Solid Waste Management

1. Introduction

Ahmedabad is the largest city and former capital of the western Indian state of Gujarat. It also earns the nickname “Manchester of east”. Almost 3500 metric tons of solid waste is generated from Ahmedabad on a daily basis. Currently more than 1600 metric tons of waste is collected under the “Door or Gate to dump project” and transported to processing plant/landfield. The solid waste fieldland site at Pirana has been operational since 1980 and about 175 lakhs metric tons of solid waste has been accumulated since then. Since October 2009 Ahmedabad Municipal Corporation has operationalized a scientific fieldland site at Gyaspur with a capacity of 11.5 lakhs metric tons.

AMC entered into an agreement with Excel Industries Ltd. since 1997 for the management of 500 tons of domestic solid waste per day through composting which requires about 25 to 30 days of processing.

Municipal Solid Waste (MSW), also called Urban Solid Waste is a waste type that includes predominantly household waste (domestic waste) with sometimes the addition of commercial wastes, construction and demolition debris, sanitation residue, and waste from streets collected by a municipality within a given area. They are in either solid or semisolid form and generally exclude industrial hazardous wastes. MSW can be broadly categorized into five broad categories (a) Biodegradable waste: food and kitchen waste, green waste (vegetables, flowers, leaves fruits), paper (can also be recycled). (b) Recyclable material: paper, glass, bottles, cans, metals, certain plastics, etc (c) Inert waste: construction and demolition waste, dirt, rocks, debris. (d) Composite waste: waste clothing, tetra packs, waste plastic such as toys. (e) Domestic hazardous waste (also called “household hazardous waste”) & toxic waste: medication, e-waste, paints, chemicals, light bulbs, fluorescent tubes, spray cans, fertilizer and pesticide containers, shoe polish.

The solid waste expresses highly diversified nature at physicochemical and biological aspects which is highly influenced by socioeconomic localities [1]. The microbial diversity studies are important in order to understand the microbial ecology in the ecosystem. The microbial community remains one of the most difficult to characterize because of their immense phenotypic and genotypic diversity. The term “diversity” as used today, spans from a molecular to a global level of biological organization and defined as “the variety of species in ecosystems, as well as the genetic variability within each species” and it is therefore the range of significantly

different kinds of organisms and their relative abundance in natural assemblage and habitat. The biodiversity can be regarded as the amount and distribution of individual species information in a natural community and thus a representative estimate of microbial biodiversity is a prerequisite for understanding the functional activity of microorganisms in ecosystem [2].

At present there is a particular interest in the relation between biodiversity, simply defined as the quality and quantity of a microbial species present in the particular ecosystem and their function in there off. The tacit assumption in many current studies are that (a) by characterizing biodiversity one can be able to understand and manipulate the working of ecosystems and (b) the ability of an ecosystem to withstand serious disturbances may depend in part on the diversity of the system.

The importance of biodiversity in the functionality of ecosystems was stressed by Agenda 21, a document from the United Nations Conference on Environment and Development, prepared in Rio de Janeiro in 1992. The document promoted scientific and international co-operation for a better understanding of the importance of biodiversity and its functions in ecosystems. There is now a growing body of experimental evidence that most organisms are functionally redundant and that the functional characteristics of component species are at least as important as the number of species per se for maintaining essential processes [3, 4]. We believe that at least some minimum number of species is essential for ecosystem functioning under steady conditions and that a large number of species is probably essential for maintaining stable processes in changing environments.

Management of solid waste reduces or eliminates adverse impacts on the environment and human health and supports economic development and improved quality of life. A number of processes are involved in effectively managing solid waste. These include monitoring, collection, transport, processing, recycling, incineration, landfilling and composting.

Composting is a stabilization process of aerobic decomposition which has been widely used for different types of waste [5]. It has been defined as intense microbial activity leading to complete or partial degradation of variety of chemical compounds of domestic solid waste by means of metabolic activity of microbial consortium. Microbial diversity is a prerequisite for a satisfactory composting process. The microorganisms needed for composting are found in compost feedstock, which can maintain an active microbial population during composting [6]. A large variety of mesophilic, thermotolerant and thermophilic aerobic microorganisms predominantly bacteria, actinomycetes, yeasts and fungi are involved in the specialized biodegradation process [7].

The process of composting occurs into three phase. (a) the mesophilic phase, (b) the thermophilic phase, which can last from a few days to several months and (c) the cooling and maturation phase. The length of the composting phases depends on the nature of the organic matter being composted and the efficiency of the process, which is determined by the degree of aeration and agitation. At the start of composting the mass is at its ambient temperature and usually slightly acidic. Soluble and easily degradable carbon sources, monosaccharides, starch and lipids are utilized by microorganisms in the early stage of composting. The pH decreases because organic acids are formed from these compounds during degradation. In the next stage microorganisms start to degrade proteins, resulting in the liberation of ammonia and increase in the pH. After the easily degradable carbon sources have been consumed, more resistant compounds such as cellulose, hemicellulose and lignin are degraded and transformed into humic acid, fulvic acid and phenolic intermediate metabolites [8]. The humified substances are divided into following groups: humin (not soluble in water at any pH), humic acids (soluble in water under alkaline conditions) and fulvic acids (soluble in water under all pH conditions) [9]. The humification of biocompost is a result of complex symbiotic and synergetic microbial interaction finally resulted into humifying earthy fragrances to an ideally compost. Of particular interest, manure and composts have received much interest and their positive impact on soil structure stability, nitrogen and carbon content [10].

The aim and objective of this research is to study the microbial biodiversity of municipal solid waste. The attempts were made to characterize the physicochemical properties of AMC domestic solid waste, and biodiversity of there off. The predominant microbial floras were studied for their catabolic profile and responsible enzymatic potency of dominant isolates.

2. Materials and methods

The AMC monitoring the domestic solid waste under the project called “Door or Gate to dump site” and being transported on the fieldland sites at Pirana and Gyaspur. The fresh raw garbage solid waste was collected from transport vehicle reaching from various areas of city to the dumping site.

The mechanical screening was carried out for the removal of nonorganic elements. The soil and mud samples were collected from the bottom of the developed waste pile from the depth of 10 cm. The water sample was collected from oozing stream nearby the developed waste pile. The biodegraded discarded residual contents from hotels, fruit market and vegetable market garbage were also selected as a domestic solid waste.

100 grams of each selected sample were collected in pre sterilized polythene bags and were preserved at refrigeration temperature in laboratory.

10 grams of each selected sample was characterized for their pH value and moisture content and then dried to prepare 300 mesh powder forms. The samples were characterized for their total carbon [11], total nitrogen [12] and C/N ratio as well as for their physicochemical nature at qualitative and quantitative organic content by means of standard analytical techniques [13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 and 24].

1.0 gram of each sample was aseptically transferred into 100 ml of 0.1% (w/v) sterile sodium pyrophosphate solution [7] into 250 ml conical flask individually. The prepared flasks were kept on orbit environment flask shaker at 150 rpm for 30 min for homogenize 10^{-2} diluted suspension. Serial dilution of 10^{-3} to 10^{-7} were prepared by subsequent aseptically transferring 1.0 ml of 10^{-2} flask suspension into 9.0 ml of sterile 0.1% sodium pyrophosphate solution tubes.

The quantitative microbial count of the selected samples were determined using 10^{-6} dilution, under the observation through 100X objective of trinocular research microscope using improved double ruled Neubauer's glass slide [25].

For the determination of qualitative and quantitative microbial community, 1.0 ml of the prepared dilution of 10^{-5} , 10^{-6} and 10^{-7} were aseptically inoculated in triplicate into various culture media agar plates specifically formulated for the cultivation of various types of bacteria, actinomycetes, yeasts and fungi [26]. The prepared 10^{-5} , 10^{-6} and 10^{-7} dilutions were also individually inoculated into various culture media agar plates supplemented with selected nutrient to determine catabolic profile of the isolates. The inoculated plates were incubated at 32°C for 48 hours to 8 days. After incubation the cultivated microbial colonies were characterized for their morphological and cytological properties and the spectrum of their nutritional catabolic profile.

3. Results and discussion

The physicochemical properties of the selected samples are highly diversified in nature (Table 1a and 1b). The fresh raw garbage solid waste collected from transport vehicle individually from Pirana and Gyaspur fieldland sites, after screening consist various sized pieces of refused residual organic material with appropriate content of moisture, organic carbon, nitrogen content and C/N ratio which are similar to physicochemical properties of solid waste of other metropolitan cities [1, 27], standardized as the appropriate properties to be a ideal matrix for composting process.

The selected fieldland sites of Pirana and Gyaspur were under operation since year 1980 and 2009 respectively, where spontaneous biodegradation of dumped materials occurred due to their native microbial community. The soil samples and mud samples of both the sites have analytical composition with reference to their organic carbon, nitrogen content and C/N ratio which indicates partial or almost biodegradation with comparison to the physicochemical analysis of ideally biocompost solid waste materials [1]. Similarly the collected water sample had similar C/N ratio with around 50% quantitative content of organic carbon and nitrogen value, as it consist only the solubilised fraction of spontaneously biodegraded solid waste matrix.

The qualitative and quantitative physicochemical analysis of hotels, fruit market and vegetable market garbage samples represent appropriate value of C/N ratio, qualifying them as suitable material for composting as well as the higher quantitative content of organic carbon and nitrogen value and its incorporation into solid waste complex could play a significant role in potent sustainable composting process (Table 1a.)

The organic carbon, nitrogen content and C/N ratio of fresh raw garbage solid waste of Pirana and Gyaspur were appropriate and competent to the data of other cities of India [28] and global standardization [1, 29] to qualify them as ideal organic matrix for potent biocompost processing. The qualitative and quantitative chemical analysis of soil, mud and water sample of both the dumping fieldland sites indicates partial spontaneous composting process during which the solid waste matrix was bioconverted into a solubilised form and qualifying as an assessable bionutrient for holophytic nutritional pattern.

Table 1a: Physicochemical properties of selected AMC domestic solid waste.

Sites	No.	Texture	Temp. (°C)	pH	Moisture %	Total Carbon Content %	Total Nitrogen Content %	C/N Ratio
Pirana	1	Fresh raw garbage solid waste: pieces of paper, textile and noncarbonic material	32.5	6.8	26.00	29.07	0.79	36.79
	2	Soil: compact brownish to dark brown	35.2	6.5	25.80	22.87	0.81	28.23
	3	Mud: compact heterogeneous dark brown to black	39.6	6.3	39.40	26.16	0.96	27.25
	4	Water: Dark brown to black slurry	34.1	5.8	92.30	12.16	0.46	26.43
Gyaspur	1	Fresh raw garbage solid waste: pieces of paper, textile and noncarbonic material	32.1	6.8	27.10	40.60	1.09	37.24
	2	Soil: compact brownish to dark brown	35.5	6.6	28.40	27.17	0.93	29.21
	3	Mud: compact heterogeneous dark brown to black	40.0	6.2	42.9	29.05	0.99	29.34
	4	Water: Dark brown to black slurry	33.8	5.7	93.05	13.05	0.46	28.36
Hotel Garbage	1	Heterogeneous solid to semisolid: Foul odoured food waste	33.3	7.4	27.2	79.49	1.96	40.55
Fruit Market Garbage	1	Heterogeneous solid: deteriorated Fruit matrix	32.1	6.1	28.1	48.75	1.58	30.85
Veg. Market Garbage	1	Heterogeneous solid: deteriorated Vegetable matrix	32.3	6.8	27.8	46.26	1.32	35.04

The Table 1b represent the qualitative and quantitative content of individual organic nutrients of the selected sample. At Pirana fieldland site the fresh raw garbage solid waste consist 0.12% soluble fraction which includes sugars, amino acids etc and 73.78% non soluble organic content including carbohydrates, proteins, lipids etc. The soil sample consist 0.27% soluble fraction and 66.88% insoluble fraction. The mud sample consist 0.44% soluble fraction and 59.11% insoluble fraction. The water sample consist 0.61% soluble fraction and 2.15% insoluble fraction.

Similarly at Gyaspur fieldland site the fresh raw garbage solid waste consist 0.09% soluble fraction which includes sugars, amino acids etc and 71.89% non soluble organic content including carbohydrates, proteins, lipids etc. The soil sample consist 0.14% soluble fraction and 65.23% insoluble fraction. The mud sample consist 0.28% soluble fraction and 56.34% insoluble fraction. The water sample consist 0.43% soluble fraction and 1.82% insoluble fraction.

The qualitative and quantitative chemical analysis of garbage samples of hotels, fruit market and vegetable market, the C/N ratio are 40.55, 30.85 and 35.04 respectively, qualifying them as a suitable material for

composting. Over and above the high content of soluble fraction 2.42%, 7.93% and 3.25% of the respective samples also enriched the initial nutritive value of complex cocktailed solid waste material for the proliferation of microbial community and enhancing the biocompost process.

Table 1b: Physicochemical properties of selected AMC domestic solid waste.

Sites	No.	Texture	Gram percentage of various nutrients in selected AMC domestic solid waste									
			Cp.A	Cp.B	Cp.C	Cp.D	Cp.E	Cp.F	Cp.G	Cp.H	Cp.I	Cp.J
Pirana	1	Fresh raw garbage solid waste: pieces of paper, textile and noncarbonic material	0.09	0.03	0.89	35.92	9.04	23.20	0.40	0.75	2.76	0.82
	2	Soil: compact brownish to dark brown	0.16	0.11	0.49	33.23	8.63	22.00	0.32	0.45	1.20	0.56
	3	Mud: compact heterogeneous dark brown to black	0.26	0.18	0.32	27.74	7.90	21.52	0.28	0.20	0.80	0.35
	4	Water: Dark brown to black slurry	0.41	0.20	0.20	0.93	0.43	0.20	-----	-----	0.34	0.05
Gyaspur	1	Fresh raw garbage solid waste: pieces of paper, textile and noncarbonic material	0.07	0.02	0.85	36.22	8.72	22.08	0.36	0.60	2.30	0.76
	2	Soil: compact brownish to dark brown	0.09	0.05	0.43	34.20	7.01	21.40	0.30	0.36	1.09	0.44
	3	Mud: compact heterogeneous dark brown to black	0.17	0.11	0.34	27.90	6.23	20.67	0.25	0.17	0.50	0.28
	4	Water: Dark brown to black slurry	0.28	0.15	0.18	0.95	0.30	0.19	-----	-----	0.17	0.03
Hotel Garbage	1	Heterogeneous solid to semisolid: Foul odoured food waste	1.12	1.30	7.61	28.20	6.18	7.60	0.12	0.32	16.8	0.85
Fruit Market Garbage	1	Heterogeneous solid: Detoriated Fruit matrix	2.12	5.81	0.32	24.60	9.21	6.30	-----	15.39	2.34	0.15
Veg. Market Garbage	1	Heterogeneous solid: detoriated vegetable matrix	1.38	1.87	4.68	21.05	11.05	9.71	-----	16.21	2.83	0.14

Note: Cp:Compound, A:Hexose, B:Compound Sugar, C:Starch, D:Cellulose, E:Hemi-cellulose, F:Lignin, G:Chitin, H:Pectin, I:Protein, J:Lipid.

Table 2: Catabolic properties of microbial community of solid waste samples (10^{-5} dilution).

Sr. No.	Sample	Microbial total count microscopically 10^{-6} dilution under 100X	Viable count N-Agar plate	Amylo-lytic colonies	Cellulo-lytic colonies	Ligninolytic colonies		Pectino-lytic colonies	Proteo-lytic colonies	Lipido-lytic colonies	Chitin-lytic colonies
				Starch Agar plate	Cellulose Agar plate	Tannin Agar plate	Lignin Agar plate	Pectin Agar plate	Gelatin Agar plate	Tributyryn Agar plate	Chitin Agar plate
1.	Pirana fieldland: Mud Sample	Protozoa: 7-8 Yeast: 5-9 Fungi: 17-22 Bacteria: 20-30	328	140	86	41	12	07	18	10	05
2.	Gyaspur fieldland: Mud Sample	Protozoa: 5-6 Yeast: 5-9 Fungi: 18-20 Bacteria: 19-25	352	129	75	46	14	09	22	06	03
3.	Hotel garbage	Protozoa: 4-5 Yeast: 7-10 Fungi: 19-25 Bacteria: 28-34	558	196	18	06	02	15	138	21	01
4.	Fruit market garbage	Protozoa: 3-5 Yeast: 11-16 Fungi: 24-28 Bacteria: 32-38	418	137	62	14	03	75	78	13	00
5.	Vegetable market garbage	Protozoa: 2-6 Yeast: 6-10 Fungi: 20-25 Bacteria: 30-35	368	176	85	25	09	48	62	10	00

At microscopic examination for total count of microbial community of selected sample with 10^{-6} dilution, the presence of various microbes were reported which includes helminthes, protozoa, yeasts, fungal spores of various fungi, Gram's negative bacteria, Gram's positive vegetative cells of spherical and rod shaped and bacterial spores (Table 2). The count of individual categories of microbes found varying with reference to the physicochemical properties of selected samples.

The quantitative microbiological analysis of fresh raw garbage solid waste had yield 182×10^5 CFU/g in case of Pirana sample and 168×10^5 CFU/g in case of Gyaspur sample which includes accounts of native microbial flora of garbage and aerial contaminants occurred during collection and transportation. The microbial counts of soil, mud and water samples of Pirana fieldland site were respectively 258×10^5 CFU/g, 328×10^5 CFU/g and 356×10^5 CFU/g. similarly the microbial count of soil, mud and water sample of Gyaspur fieldland site were respectively 237×10^5 CFU/g, 352×10^5 CFU/g and 321×10^5 CFU/g. The increased number of CFU/g in case of all these samples indicates the spontaneous composting and enrichments and potentials of catabolic profile of the responsible microbial community with reference to physicochemical nature of the sample at the various stages of compost process.

The qualitative and quantitative microbial analysis of hotels, fruit market and vegetable market garbage samples were having 558×10^5 CFU/g, 418×10^5 CFU/g and 368×10^5 CFU/g microbial count respectively which were quantitatively higher index compared to those of mud samples. The higher quantitative count of these samples was due to its more assessable native nutrient value (Table 2).

The aim of the work is to select competent, potent microbial strains for efficient sustainable composting of AMC domestic solid waste and thus the detailed investigation was focused for the microbiological characterization and screening program of microbial strains with potential catabolic nutritional pattern from the mud sample collected from Pirana and Gyaspur fieldland sites.

The quantitative microbiological evaluation of both the mud sample of Pirana and Gyaspur fieldland sites, hotels, fruit market and vegetable market garbage samples had resulted highly diversified microbial community on specialized culture media enriched with different nutritional compound which includes starch, cellulose, hemicellulose, lignin, chitin, pectin, protein and lipid. The catabolic potency of the cultivated microbes was determined by detecting the zone of utilization or treating the media plate with appropriate reagent (Figure 1) [30].

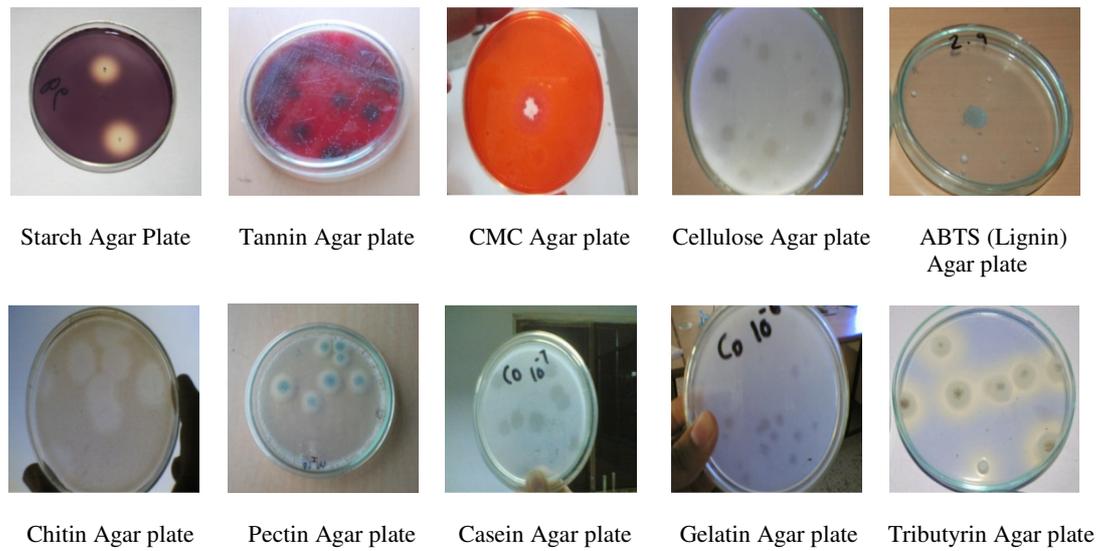


Figure 1: Catabolic reaction of isolates with selective substrate.

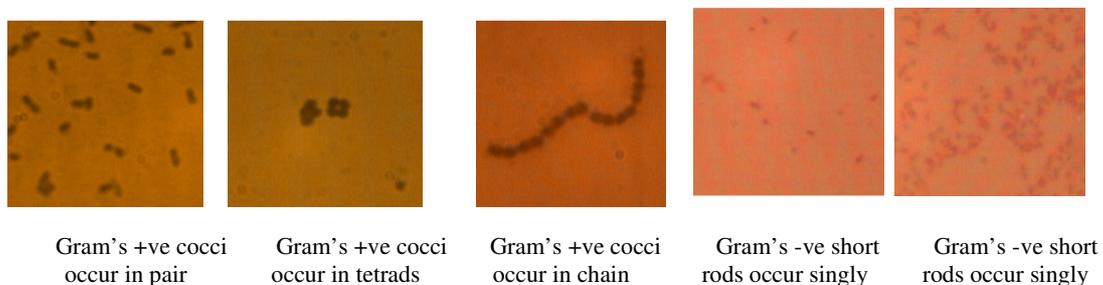
The microscopic studies of cultivated bacterial culture expressed various morphological diversified Gram's positive spherical bacterial cells with different types of cell arrangement, Gram's negative short rod found singly or in pair and rod shaped aerobic non spore forming and spore forming bacteria with different size and shape found singly or in short and long unbranched chain.

The actinomycetes- filamentous prokaryotes with highly diversified morphology were also reported in all of the selected samples. Among the cultivable actinomycetes: *Nocardia*, *Actinomyces*, *Micromonospora*, *Microployspora* and *Streptomyces* were predominant [31]. Quantitatively the mud samples were having higher counts of actinomycetes in compared to other selected samples.

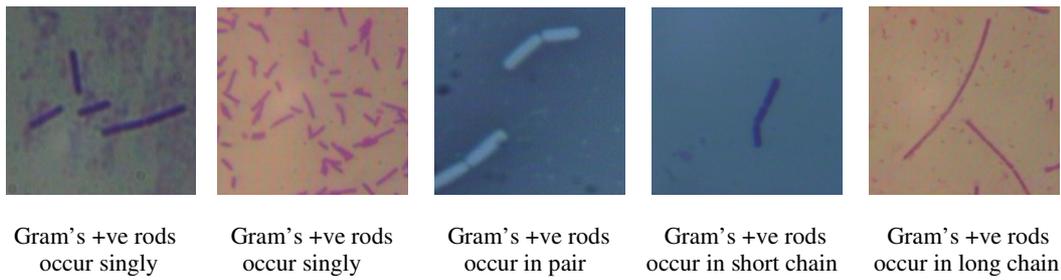
All the selected samples consist various types of unicellular, uninucleated eukaryotic yeast cells. The cultivated yeast flora exhibits various size ranging from 4 μ to 7 μ and in case of filamentous yeast with the length of 11 μ , exhibits various shape ranging from spherical, ovoidal, cylindrical to filamentous form and asexual multiplication by cell division through fission or budding which includes various pattern of polar and lateral budding [32].

All the samples consist of various types of cultivable fungi with highly diversifying morphological characters and categorized into various genera of Eumycetes. Among these fungi: *Pythium*, *Mucor*, *Rhizopus*, *Tricomycetes*, *Dimergeris*, *Neurospora*, *Aspergillus*, *Penicillium*, *Trichoderma*, *Fusarium*, *Sercospora*, *Curvularia*, *Helminthosporium*, *Episporium*, *Hetersporium*, *Alternaria* were observed predominant [33, 34].

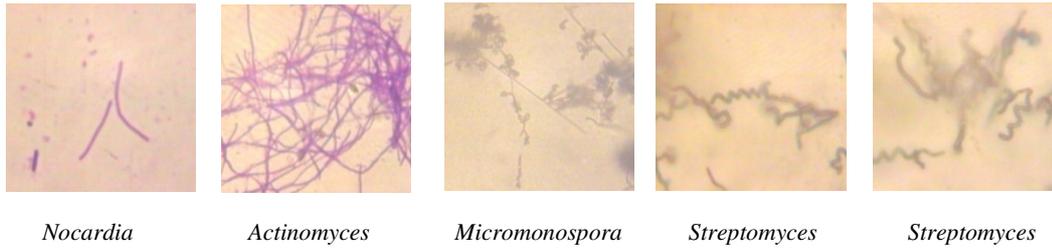
Considering the aims and objective of the work, main focus was carried out for the screening and characterization of various strains of *Aspergillus* with their catabolic profile (Figure 2 and Table 3).



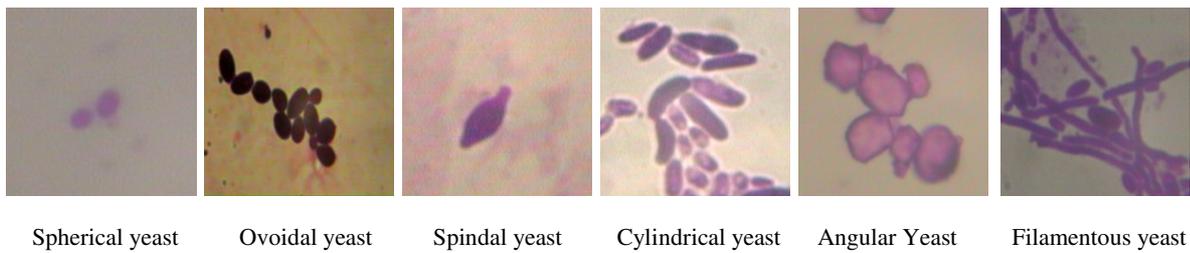
(a)



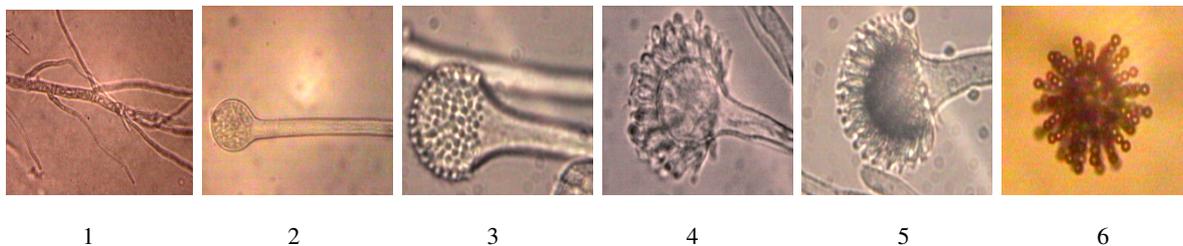
(b)



(c)



(d)



1. Filamentous septate hyphae. 2. Aerial hyphae forms terminal spherical to oval shaped swollen provesicle, native nucleus divides and forms numerous daughter nuclei. 3. In provesicle the nuclei migrate to the peripheral surface leaving central portion vacant, forming vesicle. 4. The vesicle gives budding into which the nucleus migrates forming sterigmata. 5. The sterigmata give budding forming unicellular, uninucleated, spherical, non-motile exospores called conidia. 6. The formed conidia give subsequent budding and develop chain of conidia.

(e)

Figure 2: Photographs of cultivated microbial community (a) Gram's +ve spherical bacteria and Gram's -ve short rods (b) Gram's +ve aerobic rod shaped bacteria (c) Gram's +ve filamentous bacteria (Actinomycetes) (d) Unicellular eukaryotic yeast (e) Morphogenesis of selected strain of *Aspergillus*.

Table 3: Catabolic profile of selected strains as off *Aspergillus*.

Sr. No.	Strain Category	Strain Code	Zone of utilization (mm) of selected substrate						
			Starch	Gelatin	Pectin	CMC	Tannin	Lignin	Tributyryn
1.	KA-Amy	KA-Amy 1	+++	++	+++	++	++	+	+
		KA-Amy 2	+++	++	+	++	+	+	++
		KA-Amy 3	+++	+	+	++	++	++	+
2.	KA-Pro	KA-Pro 1	++	+++	++	++	+	+	+
		KA-Pro 2	+	+++	+	+	+	+	+
		KA-Pro 3	++	+++	+	+	+	+	+
3.	KA-Pec	KA-Pec 1	++	+	+++	++	+	+	+
		KA-Pec 2	+++	+	+++	+++	++	+	+
4.	KA-Cell	KA-Cell 1	++	+	++	+++	++	++	+
		KA-Cell 2	++	+	++	+++	+++	++	+
		KA-Cell 3	++	+	++	+++	++	++	+
		KA-Cell 4	+++	+	++	+++	++	++	+
5.	KA-Tan	KA-Tan 1	++	+	++	++	+++	++	+
		KA-Tan 2	++	+	+	++	+++	++	+
6.	KA-Lig	KA-Lig 1	++	+	++	+++	+++	+++	+
		KA-Lig 2	++	+	++	+++	+++	+++	+
		KA-Lig 3	+	+	+	++	+++	+++	+
		KA-Lig 4	++	+	+	+++	+++	+++	+
7.	KA-Lip	KA-Lip 1	++	+	++	+	+	+	+++
		KA-Lip 2	++	+	++	+	+	+	+++

Note: Diameter of utilization of Zone size: +ve: 3mm to 5mm, ++ve: 6mm to 9mm, +++ve: 10mm to 15mm.

Conclusion

The physicochemical analysis of AMC domestic solid waste qualified the content as ideal matrix for composting. The physicochemical and microbiological qualitative and quantitative analysis of dumped solid waste at both the fieldland sites proved partial spontaneous to almost biocompost due to long time span of accumulation, the mud samples of both the fieldland sites consist comparatively higher microbial population and wide diversified microbial community with wide nutritional catabolic profile. The screened microbial strains with highly diversified nutritional catabolic profile could work out for secondary screening and strain development for potential, sustainable, ecofriendly, composting process.

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