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Research Article

MONITORING OF MICROBIAL POPULATION AND THEIR ACTIVITIES DURING COMPOSTING OF ORGANIC MUNICIPAL SOLID WASTES AT CENTRAL INDIA

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ABSTRACT: Seasonal changes in microbial population and the activities of cellulolytic enzymes were investigated during the composting of municipal solid waste at ranital, Central India. The changes in temperature, pH and Carbon/nitrogen (C/N) ratio were also monitored. The results obtained showed that the temperature of the windrows in all seasons reached the maximum after 3 weeks composting and then decreased by the end of the composting period (35 days), but did not reach ambient temperature. Marked changes in pH values of the compost in all seasons were found, but generally, the pH was near neutrality. Significant increases in the size of the microbial population were obtained in autumn and spring seasons compared to summer and winter seasons. The activities of cellulose were also higher in the autumn and spring seasons than in the summer and winter seasons. The decrease in C/N ratio in autumn and spring was higher than in summer and winter. It was evident that the degradation of organic matter increased by an increase in the microflora and its cellulolytic activities.
Keywords: Cellulolytic activities, composting, microbial populations, solid wastes

INTRODUCTION

Deposition of Municipal Solid Waste (MSW) is an ancient public health problem made more pressing in the 21st century by population growth, increases in per capita waste production, scarcity of suitable location for waste disposal near cities and change in the composition of waste [1, 2]. Currently, the major methods of waste management are: a) recycling-the recovery of materials from products after they have been used by consumers, b) composting- an aerobic, biological process of degradation of biodegradable organic matter, c) sewage treatment- process of treating raw sewage to produce non-toxic liquid effluent which is discharged to rivers or sea and a semi-solid sludge, which is used as a soil amendment on land, incinerated or disposed in a landfill, d) incineration- a process of combustion designed to recover energy and reduce the volume of waste going to disposal, and e) landfill- the deposition of waste in a specially designated area, which in modern sites consists of a pre-constructed 'cell' lined with an impermeable layer (man-made or natural) and with controls to minimize emissions [3].

Composting is an environmentally less burden technology because of its recycling capability of organic wastes discharged from industrial and municipal plants or livestock farming. Recent global problems of food shortage have been caused by rising cost of chemical fertilizers, and composting at low cost has been reevaluated as an important alternative fertilizer production method. High-quality compost is produced by interaction of many organisms that have suitable properties for the composting processes. Nevertheless, little information has been reported about in situ functions and roles of individual microbes in the composting processes, because many microbes related to composting are difficult to isolate and are characterized by conventional cultivation methods [4, 5].

Few fungus and bacterial isolates have been characterized in detail, and their roles in the composting process have been understood from physiological properties. For example, fungus *Chaetomium thermophilum* isolated from municipal waste compost produces extracellular enzymes, laccases, which are essential for formation of polyaromatic humic substances associated with phenoloxidase and peroxidase. *Bacillus licheniformis*, *Trichoderma viride* and complex microorganisms such as *Trichoderma* sp., white-rot fungi, *Candida rugopelliculosa*, *Bacillus casei* and *Lactobacillus buchneri* reported so far also accelerate humification of organic wastes in composting process. These characterized isolates are essential to mature compost and are useful as inoculam to increase the humification rate of composting [6, 7].

In most cities in the developed world, green waste is collected separately from other wastes and is mechanically shredded and then composted, either alone or with other organic wastes. It is used in products such as garden mulch, organic soil amendment, garden compost and soilless-potting media. However, in Australia, the main use for the composted material is as "manufactured soil" used for field-landscaping purposes in place of natural top soil. Often, inorganic additives (e.g., sand, subsoil, fly ash) are blended with the composted material [8]. Nevertheless, the inorganic component makes up only 10-30% v/v of the final product. The product is considerably cheaper than excavated natural topsoil and is, therefore, commonly used by landscape contractors. At the lower (cheaper) end of the market are manufactured soils based on rapid composting (<4 weeks) of municipal green waste to which small amounts of subsoil, sand, and/or fly ash have been added in order to give the product a more "soil-like" appearance. At the upper end of the scale are products based on co-composting green waste with easily decomposable "activator" organic materials such as animal manures and grease trap waste/ septage for periods of up to 2-3 months. Subsoil and/or top soil and inorganic additives (e.g., river sand, fly ash) are usually blended into the compost during the maturation period. Observations suggest that more intense composting (i.e., greater heat generation) occurs when activator materials are added but it is unclear how this affects the properties of the final products. More intense decomposition and stabilization may be an advantage since a characteristic of green waste-based manufactured soils is that under field conditions they slowly decompose and therefore, lose their volume over time [9, 10 and 11].

Information of the bacterial community composition and predominant genus or species is important to know the composting performance and to develop the effective composting processes. We previously reported that the combination of a hyperthermophilic pre-treatment (HTPRT) process that consists of a special reactor for ammonia removal to prevent ammonia release from composting facilities and a windrow post-treatment (WPOT) process for maturing composts (HTPRT-WPOT) is effective [12, 13]. Because the excess ammonia in the raw materials is removed first by ammonia stripping that occurred in the HTPRT reactor, the composting materials could be matured during the WPOT process by stabilized pH condition with additional effect of the elimination of ammonia odor problems. Despite using the same cow dung materials, the rate of humic acids production in the HTPRT-WPOT process is greater than in a simple windrow composting (SWC) process, as an additional advantage of HTPRT-WPOT [14, 15]. The aim of this work was to asses' impact of microbiological and physicochemical factors on composting conditions and community structure of microbial population are quite likely important factors in the composting process.

MATERIALS AND METHODS

Municipal solid waste (MSW) were collected over 24 h periods from the town of Jabalpur in central India and manually sorted in to MSW in classified facility. After sorting, the wastes were sieved in the drum to eliminate the fraction larger than 5 cm. The collected organic raw materials was first hand sorted, shredded and then composted in trapezoidal cross section windrows 25m x 3m x 2m (length x width x height) through the composting process, operation conditions; pile size, temperature, moisture content and turning were adjusted as per methods [16].

Sampling procedure

Samples were taken weekly from each pile of compost. Each composite sample was taken from ten places a depth of 90 cm and mixed together. Samples were transferred aseptically to the laboratory in a cold box.

Physical analysis
Temperature was monitored by using Raytek infrared digital thermometer as per the standard method [17].

Chemical analysis
pH and Electrical-conductivity (EC) was determined by shaking 10 g compost in 100ml distilled water (1/10, w/v) for 30 min, both parameter was measured by digital pH and EC meter. Ash was determined in samples after drying at 105ºC and ashing at 550ºC in a muffle furnance for about 3 h. Percentage of organic matter, Total Kjeldahl Nitrogen (TKN), Total organic carbon (TOC) and C/N ratio was calculated using values of the TOC and TKN as per methods [18].

Microbiological analysis
Bacteria and fungi, both mesophilic and thermophilic, were isolated from the composting samples as described [19]. Nutrient agar and potato dextrose agar media were used for bacteria and fungi respectively. Incubation temperature were 30ºC for isolation of mesophilic and 50ºC for thermophiles. The incubation time was 3 days for mesophilic bacteria, 2 days for thermophilic bacteria and 7 days for fungi, either mesophilic and thermophilic. All microbial counts were calculated on the wet weight basis. The average number of microorganisms isolated on three plates was considered the viable cell number.

Enzymatic analysis
Ten grams of sample were transferred to a flask containing 50ml acetate buffer (0.1 M, pH 5.0). The flask was shaken at 150 rpm/min for 1 h. The flask content was clarified by filtration through cheese cloth and then 10 ml of the filtrate were centrifuged at 4ºC for 15 min at 5000 rpm/min. Subsequently, the supernatant was used for measurement of enzymatic activity. The assay of microcrystalline cellulose (Avicelase) and carboxymethylcellulase (CMCase) activity was carried out by measuring the reducing sugars by the method of [20], as described [21]. The activity of β-glucosidase was measured by using p-nitrophenyl β-D- glucoside (PNPG) as described [22].

RESULTS AND DISCUSSION

Changes in temperature and pH
Seasonal changes in temperature that occurred in the windrows during the composting period (35 days) are shown in (Table 1 and Fig 1). The outside temperature was about 33± 3ºC in the day and 23 ± 3ºC in the night in case of summer and autumn seasons, whereas it was about 22 ± 3ºC in the day and 17 ± 3ºC in the night in case of winter and spring seasons. Temperature of the windrows gradually increased from 38, 35, 31 and 33ºC and reached the maximum values 61, 64, 62 and 61ºC after 3 to 4 weeks of composting in case of summer, autumn, winter and spring seasons, respectively. Then, the temperature gradually decreased to 58, 56, 54 and 57ºC by the end of the composting period in the four seasons, respectively.

It can be seen that the changes in temperature during the composting process followed a pattern typical for many composting systems [23, 24]. The peak of temperature recorded during the composting of municipal solid waste at ranital trenching site Jabalpur, usually exceeds 60ºC Well aerated composts often attain temperature of 50-65ºC and may even reach 80ºC , because of microbial activity in the decomposing plant and animal residues such as carbohydrates and proteins [25, 26]. The overall goal of the aeration is to maintain compost temperature in the range of 50-55ºC to obtain efficient thermophilic decomposition of organic wastes [27, 28]. During the composting under the compost plant operation conditions, the aeration was not adjusted as usual and therefore the decomposition of organic wastes was very slow, especially when the moisture content was low, as in case of summer and winter seasons.

It was inferred that the course of temperature in a compost pile is indicative of the progress of the composting process from its beginning to its completion. Compost is matured enough when the temperature remains more or less constant and does not vary with the turning of the material. Therefore this parameter is considered as a good indicator for the end of the bio-oxidative phase in which the compost achieves some degree of maturity [29, 30]. During the composting process, the decrease in temperature at end of the composting period was very slow, especially in summer and winter seasons. This may be attributed to the decrease in moisture content of organic wastes and therefore the decrease in decomposition of wastes.

On the the other hand, the decrease in temperature in the case of the autumn and spring seasons was high and this may be attributed to the suitability of moisture content for microbial and enzymatic activity and therefore the increase in decomposting of wastes [31, 32]. Moisture content of compost in all seasons under the compost plant operating conditions was not adjusted as usual and it ranged: 22.7-34.0 %; 42.0-46.0 %; 35.0-42.0% and 36.0-48.0 % throughout the composting period in case of summer, autumn, winter and spring seasons, respectively as shown in (Table 1 and Fig 1).

Moisture content is critical parameters in the composting process. It influences the microbial activity, free air space, oxygen transfer and temperature of the process [33]. It was mention that biological activity can be greatly reduced at a moisture content of less than 30% [34]. For these reasons, the composting process was better in autumn and spring than in summer and winter, where the moisture content was nearly suitable but not optimum for the microbial and enzymatic activities. Moisture content of 50-60% is recommended to be the optimum condition for composting by numerous investigators [35, 36].

Table 1: Seasonal changes in temperature, moisture content, pH and C/N ratio during composting of municipal solid wastes at Jabalpur.

Parameters	**Seasons**	**Composting time in days**					
		0 days	**7 days**	**14 days**	**21 days**	**28 days**	**35 days**
Temperature	**Summer**	38.0± 0.84	49.0± 0.82	55.0± 0.90	56.0± 0.82	61.0± 0.80	58.0± 0.78
	Autumn	35.0± 0.80	50.0± 0.90	58.0± 0.90	61.0± 0.85	64.0± 0.82	56.0± 0.72
	Winter	31.0± 0.65	47.0± 0.72	56.0± 0.82	58.0± 0.68	62.0± 0.80	54.0± 0.60
	Spring	33.0± 0.80	52.0± 0.90	55.0± 0.78	56.0± 0.86	61.0± 0.72	57.0± 0.80
Moisture	**Summer**	34.0± 0.58	32.0± 0.68	27.0± 0.52	24.5± 0.56	24.3± 0.58	22.7± 0.65
	Autumn	46.0± 0.62	42.0± 0.80	51.0± 0.72	54.0± 0.66	45.0± 0.76	42.0± 0.84
	Winter	42.0± 0.80	39.0± 0.65	44.0± 0.90	40.0± 0.82	38.0± 0.58	35.0± 0.78
	Spring	48.0± 0.90	45.0± 0.82	47.0± 0.52	42.0± 0.70	38.0± 0.62	36.0± 0.80
pH	**Summer**	7.82± 0.023	7.32± 0.016	6.86± 0.025	6.52± 0.042	7.21± 0.034	7.49± 0.062
	Autumn	7.68± 0.023	7.40± 0.054	6.97± 0.031	6.65± 0.018	7.08± 0.026	7.38± 0.065
	Winter	7.26± 0.052	6.92± 0.048	6.57± 0.037	6.84± 0.056	6.58± 0.012	7.02± 0.029
	Spring	7.54± 0.90	7.08± 0.90	6.83± 0.90	6.42± 0.90	6.78± 0.90	7.25± 0.90
C/N ratio	**Summer**	42.38± 0.12	38.14± 0.24	35.08± 0.02	31.14± 0.47	28.61± 0.32	25.37± 0.52
	Autumn	38.25± 0.52	35.10± 0.68	32.24± 0.72	28.12± 0.27	25.37± 0.38	23.41± 0.19
	Winter	40.32± 0.08	36.25± 0.42	34.28± 0.92	31.58± 0.18	27.64± 0.50	24.12± 0.74
	Spring	41.59± 0.40	38.19± 0.64	35.24± 0.90	33.47± 0.52	30.15± 0.49	27.38± 0.26

Values are means ± standard errors.

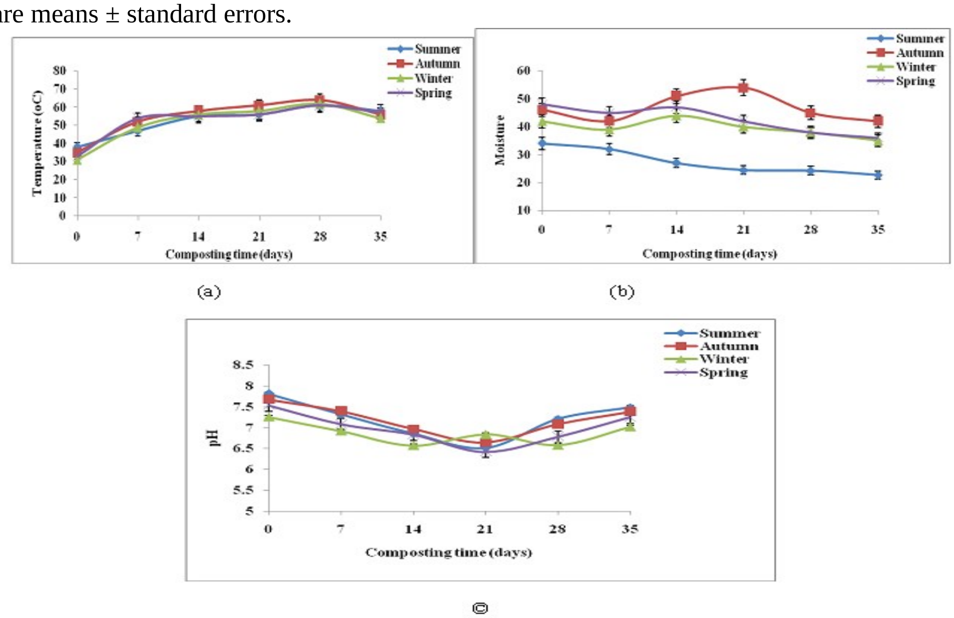
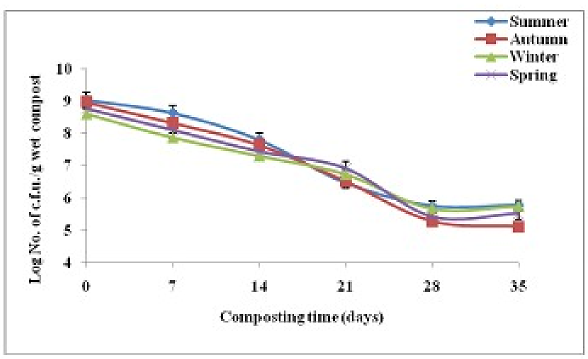


Fig 1. Seasonal changes in (a) Temperature ºC, (b) Moisture content and (c) pH during the composting of municipal solid waste at Jabalpur. Values of three replicates ± standard errors.

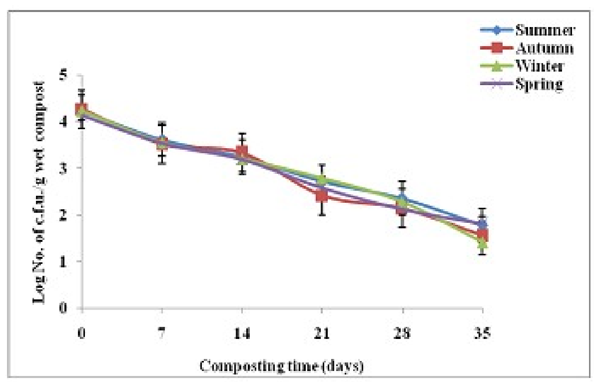
The pattern of pH during the composting process in all seasons was typical for composting processes as described by several authors [37, 38]. The average changes in ph values of compost samples are shown in (Table 1 and Fig 1). The starting pH values were 7.82, 7.68, 7.26 and 7.54 in the summer, autumn, winter and spring seasons respectively. After 2 weeks of composting, the pH decreased to 6.68, 6.97, 6.57 and 6.83 and then gradually increased by the end of composting to 7.49, 7.38, 7.02 and 7.25 in case of summer, autumn, winter and spring seasons respectively. It is noticed that the decrease in pH values, delayed (after 2 weeks) and this could be attributed to the conditions of composting which were not adjusted usual. The slight alkalinization occurred as soon as the mass temperature increased, whereas during the cooling down it decreased to near the neutral value and then stabilized. The decrease in pH during the first period of composting is expected because of the acids formed during the metabolism of readily available carbohydrates. After this initial stage, the pH is expected to rise, with evolution of free ammonia and to stabilize or drop slightly again to near neutral as results of humus formation with its buffering capacity at the termination of composting activity [39, 40].

Changes in microbial populations

Seasonal changes in the numbers of mesophilic and thermophilic bacteria and fungi during the composting of municipal solid waste compost are illustrated in Fig 2 and 3. The figures show the logarithm of the number present. Initially, in the wastes before composting, there was a high number of mesophilic bacteria and lower number of themrmophilic ones, where low. As shown in Fig 2a, the number of mesophilic bacteria gradually decreased during the composting process in all seasons. The decrease was higher in case of summer seasons than the other seasons and it could be attributed to the decrease in moisture content in summer seasons where it was unsuitable to microbial growth, whereas, the windrows temperatures and moisture content in case of other seasons are considered to be suitable but not the optimum for composting [41, 42]. The nearest conditions to the optimum were found in autumn and spring seasons. Generally, the numbers of these bacteria were also lower in summer seasons than those in the other seasons and this may be attributed to the same reasons as mentioned above. On the other hand, the thermophilic bacteria count increased and reached the optimum numbers after 3 weeks of composting period (Fig 2b).



(a)



(b)

Fig 2. Seasonal changes in count of (a) mesophilic and (b) thermophilic bacteria during the composting of municipal solid waste at Jabalpur. Values of five three ± standard errors. * c.f.u. colony-forming units.

As shown in Fig 3a, the number of mesophilic fungi gradually decreased throughout the composting process in the four seasons and disappeared completely after 3 weeks when the temperature rose to more than 60ºC. On the other hand, the thermophilic fungi increased and reached the maximum numbers after 2 weeks of composting and then gradually decreased (Fig 3b).

A slight increase was found at the end of composting in case of the autumn and spring seasons when the temperature was decreased to 50ºC. Most fungi are eliminated at the temperature above 50ºC, only a few have been isolated from compost that can grow at all up to 62ºC. Their survival was due to their thermotolerent property. During composting, temperature above 55ºC. discourage fungal growth. Fungi are excluded from waste composting during the earlier high temperature stage [43, 44 and 45].

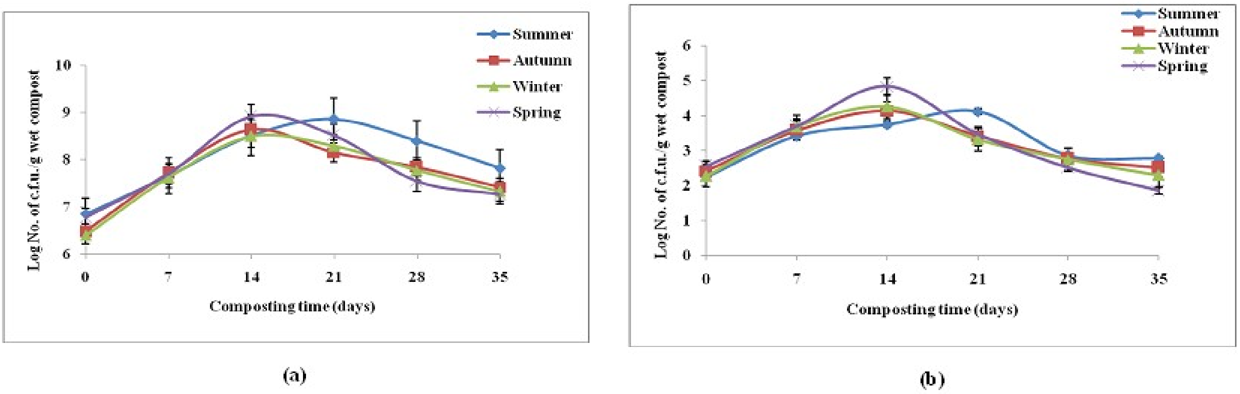


Fig 3. Seasonal changes in count of (a) mesophilic and (b) thermophilic fungi during the composting of municipal solid waste at Jabalpur. Values of five three ± standard errors. * c.f.u. colony-forming units.

From the results obtained, it can be seen that the most common microorganism 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 there are the more tolerant of high temperature. Generally, mesophilic microorganisms are responsible for the initial decomposition of organic materials and the generation of heat responsible for the increase in compost temperature. As the temperature begins to rise, thermophilic microorganisms begin to dominate, while during the cooling phase of composting, mesophilic microorganisms begin to dominate [46]. The microbial biomass of some groups of microorganisms, especially the thermophilic bacteria, decreases in the last phases of composting as the product reaches maturity. Hence a total count of microorganisms (principally bacteria) throughout the process can be indicative to the state of compost maturity [47, 48].

Changes in cellulolytic activities

Seasonal changes in activities of Avicelase, CMCase and β-glucosidase enzymes were assayed in compost extracts during the composting process. As shown in Fig 4a, the activity of Avicelase increased and reached maximum values (0.039, 0.042, 0.045 and 0.044 µmol/ml/min) after 3 weeks in the case of summer, autumn, winter and spring seasons, respectively, and then decreased by the end of the composting period. The same trend was obtained with CMCase (Fig 4b). The maximum values (0.28, 0.30, 0.26 and 0.29 µmol/ml/min) were also found after 3 weeks of composting and then gradually decreased. The activity of β-glucosidase gradually increased and reached the maximum values (3.4, 3.6, 3.3 and 3.5 µmol/ml/min) after 1 weeks of composting in case of summer seasons, autumn, winter and spring seasons, respectively and then gradually decreased by the end of composting in the case of autumn and spring seasons the activity decreased after 2 weeks and then slightly increased again after 3 weeks and continued until the end of composting (Fig 4c). It is noticed from the results obtained that the activities of Avicelase and CMCase reached a maximum as soon as the mass temperature increased. The highest activity of these enzymes was found at the highest temperature in all seasons. Therefore, the optimal conditions for cellulose activity appear to occur within the first 3 weeks of composting. These results agree with those reported [49, 50].

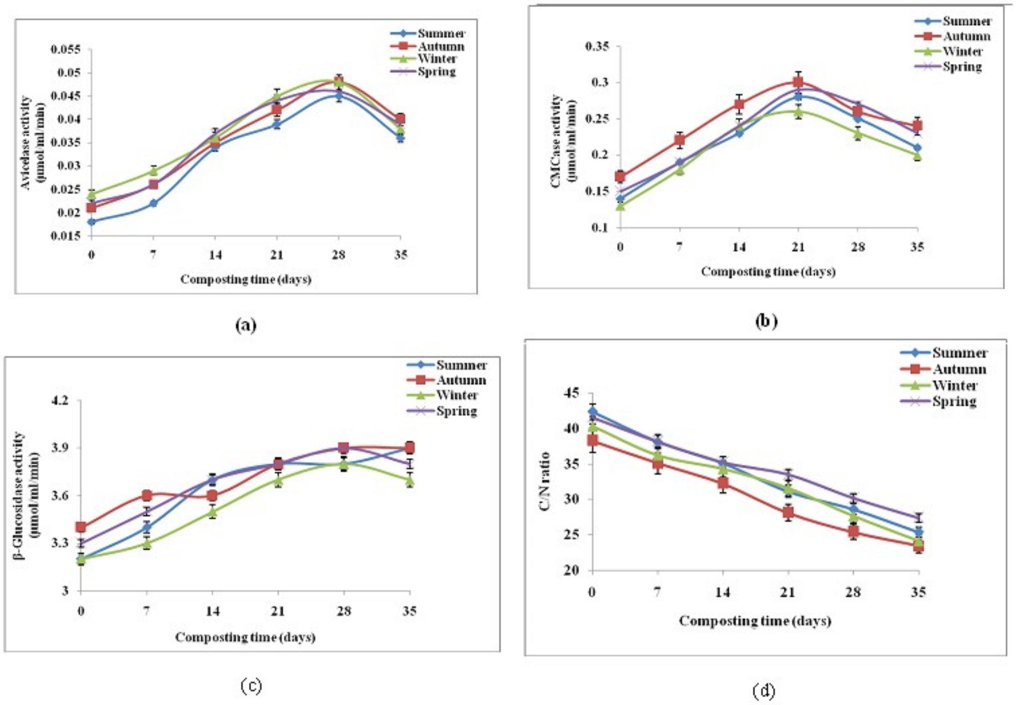


Fig 4. Seasonal changes in (a) Avicelase, (b) CMCase, (c) β-glucosidase activities of compost extracts and (d) C/N ratios during the composting of municipal solid waste at Jabalpur. Values of five three ± standard errors.

Initial low cellulose activities could be attributed to the availability of simple compounds and the subsequent higher activity at the lower C/N ratio may be attributed to the greater nitrogen availability [51, 52]. Also, the activity of these enzymes was related to the number of microorganisms, especially the thermophilic ones. It is noticed also that the activities of these enzymes were higher in case of autumn and spring seasons than in the case of summer and winter seasons and this could be attributed to the suitability of composting conditions such as temperature and moisture content for microbial and enzymatic activities. On the other hand, the reason for the increase in β-glucosidase activity after 3 weeks in the case of autumn and spring seasons could be ascribed to the increase of Avicelase and CMCase activity and therefore the availability of liberated cellobiose to the β-glucosidase enzymes [53, 54].

Changes in C/N ratio

The seasonal changes in C/N ratio during the composting process are illustrated in Table 1 and Fig. 4d, The decreased in C/N ratio in the case of the autumn and spring seasons was higher than in summer and winter seasons. This could be attributed to the increase of microbial population and their cellulolytic activities when the moisture content and temperature were nearer the optimum for composting, in the case of the autumn and spring seasons. On the other hand, in the case of summer and winter seasons the temperature was suitable but the moisture content was not suitable for composting and therefore the microbial populations and their cellulolytic activities were low.

The C/N ratio is often used as an index of compost maturity, despite many pitfalls associated with this approach [55], but it seems to be a reliable parameter for following the development of the composting process. The present data showed that the C/N ratio in the final compost was 25.37, 23.41, 24.12 and 27.38 in the case autumn, winter and spring seasons, respectively. These ratios are considered to be high especially in the autumn and winter seasons therefore, these composts are not matured. It has been stated that the C/N ratio of matured compost should ideally be about 10 but this is hardly ever achievable, due to the presence of recalcitrant organic compounds, or materials which resist decomposition due to their physical or chemical properties [56].Some authors reported that a C/N ratio below 20 is indicative of an acceptable maturity, a ratio of 15 or even less being preferable [57].

CONCLUSION

Generally, the compost obtained during the autumn and spring seasons according to the decrease in C/N ratio and the decrease in temperature at end of composting and this could be attributed to the suitability of moisture content with temperature for microbial populations and their cellulolytic activities. From this study, it can be concluded that the adjustment of composting conditions such as pile size aeration, moisture content and temperature is very important. This would allow from microbial populations and their enzymatic to increase and therefore the increase of organic material decomposition as a reduction in C/N ratio and then the composting time can be reduces. Therefore, composting could be easily as appropriate techno logy for India to produced a useful product and reduce the environment pollution if the optimum conditions are ensured.

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