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Full Length Research Paper

Vermicomposting of vegetable waste: A bio-physicochemical process based on hydro-operating bioreactor

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The increasing waste generation rate, high collection cost and dwindling financial resources are the major problems faced by most of the developing countries for efficient solid waste management. In some cities, the organic waste (market, municipal, household) are dumped indiscriminately or littered on the streets causing environmental deterioration. Biological processes such as composting followed by vermicomposting to convert vegetables waste (as valuable nutrient source) in agriculturally useful organic fertilizer would be of great benefit. Therefore this technique is studied in the present research work. A simple and potentially inexpensive Hydro Based Operating Bioreactor (HBOB) was developed for aeration and turning of plant biomass for efficient aerobic composting process. The composting process was done viz ambient, mesophilic, thermophilic and cooling down stages for a period of two weeks. After cooling stage, the partially decomposed material was used further as bedding for earthworms for vermicomposting. Experiments were conducted to determine the changes in the physicochemical parameters (pH, temperature, moisture content and C/N ratio). The dominant species of microorganisms at different temperatures during entire process of composting and vermicomposting were investigated. Self heating of the ingredients due to microbial activity occurred within the bioreactor at the thermophilic stage of the composting process. The vermicompost developed in the HBOB was found to have comparatively high value of nutrients such as calcium, sodium, magnesium, iron, zinc, manganese and copper which can serve as a natural fertilizer giving high yield of plants. The vermicomposting has proved very effective and efficient for developing compost from vegetable waste.

Key words: Waste management, composting; bioreactor, vegetable waste, earthworms, physico-chemical characterization.

INTRODUCTION

In India, about 320 million tones of agricultural waste are generated annually (Suthar et al., 2005) of which vegetable waste alone is in major proportion. The waste from the vegetable market is collected and dumped into the municipal land fills, causing a nuisance because of high biodegradability (Bouallagui et al., 2004). This result in loss of potentially valuable materials that can be processed as fertilizer, fuel and fodder (Baffi et al., 2005). The biological treatment of these wastes appears to be most cost effective and carry a less negative environmental impact (Coker, 2006; Paraskeva and Diamadopoulos, 2006). A possible way to utilize this waste is by vermicomposting biotechnology (Benifez et al., 1999; Mills, 2006), followed by aerobic composting in an aerobic bioreactor. In recent years, a number of novel reactor designs have been adopted and developed (Nakasaki and Ohtaki, 2002; Petiot and Guardia, 2004; Smith et al., 2006), but they have few limitations like high investment, operating and handling costs (Bohn et al., 2007). To take full advantage of the market potential of this waste, innovative systems are needed.

We have proposed a new approach by developing a Hydro Based Operating Bioreactor (HBOB). The design-

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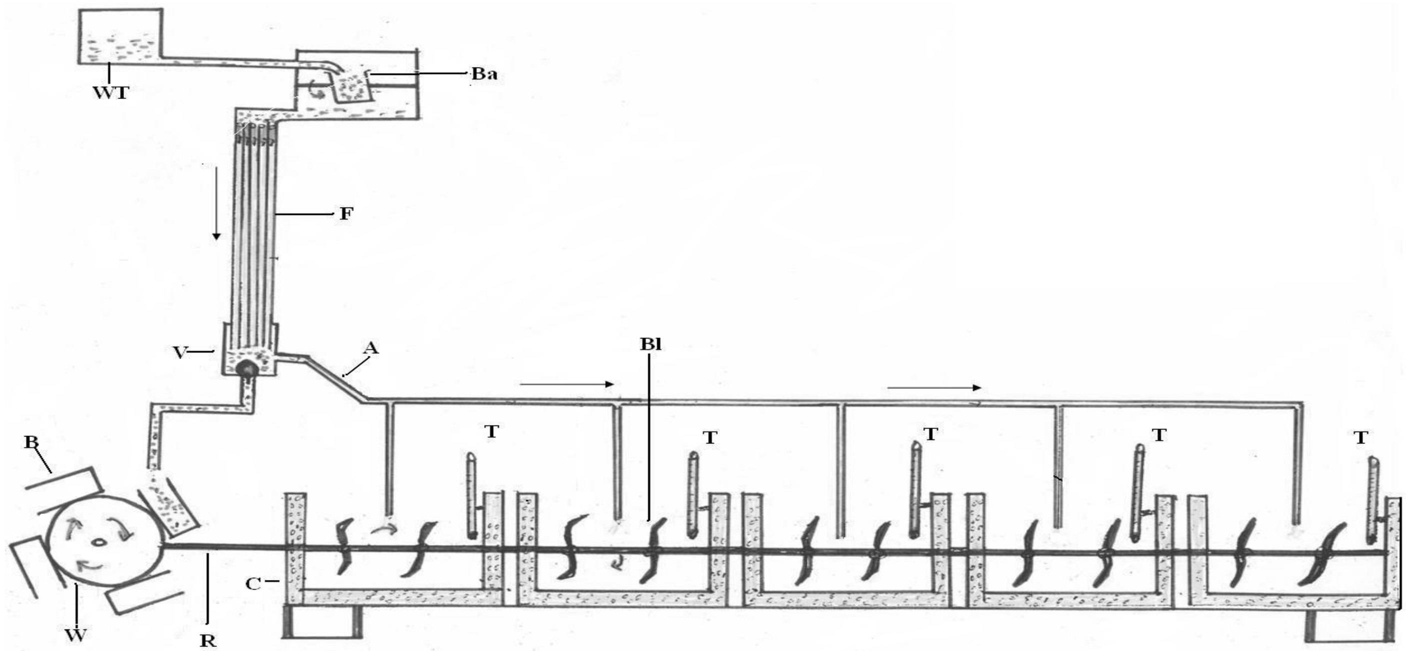

Figure 1. Schematic diagram of hydro based operating bioreactor. A, Air tube; B, Box; Ba, Balance box; Bl, Blade; C, Perforated Plastic container of dimension [(2(l) X 1(b) X 1 (h)] ft.; F, Fine tubes; R, Rod; T, Thermometer; V, Valve; W, Wheel; WT, Water tank.

ned bioreactor permits the air flow and turning of composting particle with the help of water to maintain aerobic condition necessary for bedding of earthworms. During the bedding period, the raw materials undergo biological oxidative decomposition by successive communities of microorganisms i.e. ambient, mesophilic and thermophilic under different temperature regimes (Butler et al., 2001). Giving ideal condition of aeration, turning of composting material in developed bioreactor as well as by maintaining pH, moisture content, particle size and C:N ratio, the process generates high temperature (55 to 68°C) for adequate period. These temperatures inactivate the harmful pathogens (Jiarg et al., 2003) and develop good bedding suitable for earthworms. Further on the partially decomposed bedding material is subjected to vermicomposting by using *Eisenia foetida*.

The vermicompost is a rich source of beneficial micro-organisms and nutrients (Paul, 2000) and is used as a soil conditioner or fertilizer (Hattenschwile and Gaser, 2005; Rock and Martnes, 1995). Increase in crop yield, soil nutrients status and nutrients uptake was reported due to application of vermicompost (Roberts et al., 2007; Singh and Sharma, 2003). The developed vermicompost from this technology has high nutrient values (offer a whole range of socio- economic benefits such as saving time, electricity, man power for farmers of the developing country). The aim of the research work is to develop an innovative HBOB to maintain aerobic condition during composting (bedding) and to study the bio-physicochemical interactions involved during the vermicomposting.

MATERIALS AND METHODS

Composting reactor and operation

In order to study the mechanism of composting, composting reactor was developed for the production of compost from vegetables waste. The reactor consists of five perforated (sides and bottom) rectangular plastic boxes which were arranged side by side having dimensions of [2 (l) x 1 (b) x 1 (h)] ft, of each box serving distribution of air supply from outside as shown in Figure 1. The working volume of each plastic box was 15-kgs. It was equipped with water based aeration system (tube column) and hydraulic stirrer system. The Tube Column (T.C.) consists of a bundle of 50 tubes each of 3.0-meter long and 5-mm inner diameter arranged vertically along the length of the column.

For the bioreactor to work, 2 liter of water was supplied from the water tank (WT) at the lower flow rate i.e. 30 min to fill the balance box (Ba), where it gets filled and rotated through 90 degree and pouring the collected water instantly at the top of the tube column. In the tube column, the water acts as a piston pushing the air forcefully in downward direction. This air then passed through the air tubes (A), connected to the container and diffuses in to the composting material to provide the necessary amount of oxygen to maintain an aerobic condition. The water in the tube column follows the air and gets collected in the box (B) of the rotating wheel (W) tangentially. The developed torque (rotational motion) rotates the rotating wheel by the weight of water. The rotating wheel functions as a driving force for the common rod (R), having a pair of blades (BL) in each box of reactor for turning of the composting particles, helping to form a good composting bed. Thus the operation of each cycle leads to aeration and mixing. The aeration was provided at a rate of 1 - 5 liter/kg/min. The temperature was detected with the thermometers (T), which were placed in each box.

Composting materials

The materials which decomposed slowly with aeration high lignin content were used for composting (bedding). Each container of the composting reactor was filled with a layer of coconut fibers, palm tree leaves up to a 1.5-inch height and the 3-inch layer of dried leaves, and fresh grass collected from the University campus. Some amount of alluvial soil, collected from Palghar (located 100 km away from Mumbai), was sprinkled on the above materials to provide grit for worm digestive system. Finally, a layer of cow dung slurry was spread. Similarly two more layers of above mentioned materials were made and the moisture content was maintained up to 55 to 65% by watering. The vegetable waste of cabbage, french bean, cauliflower, lady finger, spinach, and carrot were collected from the waste dumping site and used as basic raw material for development of compost. Suitable amount of vegetables (200 to 300 g) waste was chopped in to 2-5 mm size and spread over the composting material.

During the composting processes, microorganism's status was assessed at the ambient temperature. The mesophilic conditions started immediately after a day. Mesophilic conditions lasted for about one week at the temperature of about 40°C after which the microorganism's status was again assessed. After this, temperature at the thermophilic conditions had risen ranging from to 40 to 67°C. The thermophilic conditions were assessed for its population diversity. Further the temperature was cooled down after the thermophilic conditions, allowing composting material to stabilize and mature. Physicochemical and biological characteristics during these stages were monitored and assessed for a period of 2 weeks. The reactor was run twice a day for a period of 15 days during the mesophilic and thermophilic conditions.

Bedding material

The above partially decomposed cooled material was used as bedding for earthworm.

Adding worms

About ½ kg of exotic varieties of earthworms (*Eisenia foetida*) was spread on bedding materials. Everyday, 200 to 300 g of vegetable waste collected from market was supplied to each container for a period of two and half months as a source of food for the earthworms. The physicochemical and biological characteristics were monitored during vermicomposting. After two and half month, vermicompost was collected, air dried, sieved (2-mm) and a portion of it was taken for nutrient analysis in order to prove its potency as bio-fertilizer.

Isolation of dominant microorganisms

Bacteria, actinomycetes and fungi were isolated from vegetables waste, cow dung and soil during composting stages (i.e. ambient, mesophilic and thermophilic) and vermicomposting. Trypticase soy agar medium was used, for bacteria, [Trypticase peptone, 17 g; phytone peptone, 3 g; NaCl, 5 g; K_2HPO_4, 2.5 g; glucose, 2.5 g; agar, 20 g; distilled water, 1 litre (pH 7.3)]. The Malt-yeast extract agar medium for actinomycetes [yeast extract, 4 g; malt extract (Himedia), 8 g; glucose, 4 g; agar, 20 g; distilled water, 1 litre (pH 7.3)] and potato dextrose agar medium for fungi [PDA, 39 g; distilled water, 1 liter (pH 5.2)] (Atlas,1993).

10 g of each sample was suspended separately in 90 ml of sterile distilled water and homogenized at 10,000 rpm for 10 min with a homogenizer. By serial dilution in sterile water, the suspensions were prepared and spread on to the media plates. Then the media plates of vegetable waste, cow dung, soil, ambient staged compost and vermicompost samples were incubated at 30°C for 4 days. The incubation temperature was 37°C for isolation of mesophiles and 55 to 65°C for thermophiles. The incubation time was 3 days for mesophiles and 2 days for thermophilic bacteria according to the standard method (Nakasaki et al., 1985). The microorganism colonies from samples were identified by cellular morphology and biochemical characteristics. Dominant microorganisms were isolated from all the samples.

Analytical methods

All the samples characterization was conducted in triplicate to obtain mean values. The physicochemical parameters were measured by standard methods. The moisture content of samples was calculated by the weight difference before and after drying at 105°C to a constant weight. The pH and electrical conductivity (EC) were measured after 20 min of vigorous mixed samples at 1: 2.5 (solid : deionized water ratio) using digital meters [Elico, Model LI-120] with a combination pH electrode and a 1-cm platinum conductivity cell, respectively. Total nitrogen and total phosphorus were determined according to the standard methods of the American Public Health Association (1998). Cation exchange capacity was determined after extraction with ammonium acetate at pH 7.0, and the organic carbon was determined by using Walkley–Black method (Jackson, 1973). The nutrients in dried samples of vegetables waste, composting stages and vermicompost were digested with concentrated nitric acid and 30% hydrogen peroxide and then determined by an atomic absorption spectrophotometer [AAS, Perkin Elmer] (APHA, 1998).

RESULTS AND DISCUSSION

Composting and vermicomposting are bioxidative processes that stabilize the organic matter. Composting includes a thermophilic phase during which labile organic matter degradation occurs and pathogens are effectively reduced. Vermicomposting includes coupled activities of earthworms and microorganisms, stabilizing the organic matter and does not involve a thermophilic phase (Tognetti et al., 2005). In our study, aerobic composting was carried out using vegetable waste in combination with plant litter which further could convert into bedding material for vermicomposting. Five composting trials in five boxes of bioreactor under well controlled environmental conditions at a constant external temperature of 30°C were studied.

The composting requires sufficient amount of oxygen for aerobic activity as too little aeration leads to anaerobic conditions whereas excessive cooling may prevent the thermophilic conditions at too much of aeration (Ahn et al., 2007). In order to achieve these aerobic conditions, this self contained instrument (bioreactor) was constructed with simple design and operation. Its efficient performance has attributed to its maintained air flow at 1-5 lit/kg/min. Previous researchers have recommended a variety of aeration rate ranges to optimize the composting process. Lu et al. (2001) reported a flow rate of 0.43 - 0.86 lit/kg/min to be efficient in maintaining the thermophilic phase during the composting trials of food waste. The constructed bioreactor being hydro-based saves on energy sources like water and electricity, also making the used water reusable. During composting, the changes in

Table 1. Physicochemical analysis during composting stage of vegetable waste.

Composting Stage	pH (1:2.5 H_2O)	Temp (°C)	Total carbon (%)	Total nitrogen (%)	C/N ratio	Moisture content (%)	Water holding capacity (%)	EC ($dS\ m^{-1}$)
Ambient	6.5	30.5	36.27	1.50	24.18	65.52	41.20	1.01
Mesophilic	5.9	39.1	31.15	1. 40	22.25	64.60	44.04	5.40
Thermophilic	8.6	60.2	22.72	1.10	20.65	52.00	44.28	6.94
Cooling	7.2	33.4	20.35	1.05	19.38	50.00	45.53	7.10

Values are averages of three replicates.

Table 2. Physicochemical analysis during vermicomposting of vegetable waste.

Vermicomposting period (week)	pH (1:2.5 H_2O)	Temp (°C)	Total carbon (%)	Total nitrogen (%)	C/N ratio	Moisture content (%)	Water holding capacity (%)	EC ($dS\ m^{-1}$)
1	7.2	32.5	19.00	1.06	17.92	74.30	49.57	7.75
2	7.1	32.0	17.30	1.08	16.01	72.50	53.29	7.80
3	7.5	31.0	16.60	1.10	15.09	72.20	55.92	7.29
4	7.2	30.0	16.00	1.09	14.67	70.00	56.86	8.10
5	7.1	30.0	15.60	1.15	13.56	73.00	58.24	8.20
6	6.9	29.5	15.23	1.17	13.01	75.00	61.67	8.50
7	6.5	29.3	15.11	1.19	12.69	72.28	62.90	9.30
8	6.8	29.3	15.00	1.21	12.39	72.00	66.30	10.29
9	6.4	29.1	14.23	1.24	11.47	71.37	69.10	10.40
10	6.8	29.0	13.50	1.33	10.15	70.15	72.00	10.55

Values are averages of three replicates.

bio- physicochemical parameters were observed and are described below.

Changes in pH

The pH varied during the composting and vermicomposting process. At the ambient stage, the pH of the composting material was 6.5. As the composting process progressed at mesophilic stage the pH reduced to 5.9. In the thermophilic stage, the pH value raised rapidly to 8.6. During the cooling stage, the pH fell towards neutrality i.e. 7.2 (Table 1). The pH had a slow decrease during vermicomposting (Table 2). Composting materials varied widely in pH. Initially, many materials such as vegetables waste are acidic (Nakasaki and Ohtaki, 2002); metabolic process during composting further affect the pH of the material. The initial drop in pH at the mesophilic stage may be due to the action of microorganisms on carbohydrate, the most labile fraction of organic matter leading to the release of organic acids (Fang et al., 2001). The pH subsequently increased at the thermophilic stage probably due to the production of ammonia (Guoxue et al., 2001; Crawford, 1985). The pH remained high until the end of the composting process, possibly due to progressive utilization of organic acids and increase in mineral constituents of waste (Tufts, 1993). These observations are in conformity with those obtained by Fares et al. (2005).

Changes in temperature

The temperature was found to increase from the ambient to mesophilic stage and then thermophilic, where actual decomposition of vegetable waste occurs. At the ambient stage, the temperature of the waste was 30°C. As the composting process progressed at mesophilic stage, the temperature was raised to 39.1°C. In the thermophilic stage, the temperature was raised to 60.2°C, where it fluctuated between 56 to 42°C for the period of one week and then gradually decreased to 33.4°C at cooling stage (Table 1). The temperature maintained a gradual decline during vermicomposting period and stabilized to 29°C at the end (Table 2).

The heat released by the oxidative action of microbial activity in conversion of organic matter results in the rise in temperature during the composting process (Peigne and Girardin, 2004). Thus, at the start, the material is at ambient temperature and as the indigenous mesophilic organisms increase in number the temperature rises.

These mesophilic organisms are then succeeded by thermophilic bacteria and fungi as the temperature rises above 40°C. It is the active phase of composting where most of the organic matter is degraded and consequently most of the oxygen was consumed. According to Fang and Wong (2000), lignin degradation starts during thermophilic phase. The optimum temperature for the thermophilic micro-fungi and actinomycetes which mainly degrades lignin is 40-50^{o}C and above 60^{o}C. These microorganisms can not grow and lignin degradation becomes slow. After the thermophilic phase, which corresponds to a peak of degradation of fresh organic matter, the microbial activity decreases as does the temperature. This is called the cooling phase. The compost maturation phase begins when the compost temperature falls to that of the ambient (Zibilske, 1999).

Changes in organic carbon, nitrogen and C:N ratio

The losses of organic carbon were significantly affected by composting and vermicomposting. It was found that the percentage of organic carbon decreased from ambient to mesophilic, thermophilic, cooling down stage and during the period of vermicomposting which shows the decomposition of waste by microbial population. Thus over the course of the composting and vermicomposting trial, the concentration of organic carbon declined from 36.27 to 13.50% (Tables 1 and 2).

The organic carbon was being decomposed by the microbial biomass present in the compost (Mondini et al., 2003). Part of the carbon in the decomposing residues evolved as CO_2 and a part was assimilated by the microbial biomass (Cabrera et al., 2005; Fang et al., 2001; Nakasaki et al., 1985). Fares et al. (2005) reported that carbon loss accounted for 46 to 62% of initial total carbon during the composting process. Similarly, Guest et al. (2001) observed the decrease in carbon concentration from 20 to 16% during the composting process.

The nitrogen content of starting material was 1.50%; then it showed a rapid decrease in the early days of the composting period with maximum at thermophilic stage and then slight increase during the process of vermicomposting (Tables 1 and 2). The organic material was in forms of cellulose or lignin which is in insoluble form and the nitrogen present in the ‘lignin humus complexes’, formed by microbial activity in the composting process was not available unless lignin broke down. This in turn results into nitrogen loss (Crawford, 1985). The decrease in percentage of nitrogen concentration during the composting process due to release of ammonia has been investigated by Guest et al. (2001). The increase in nitrogen content during vermicomposting is due to the nitrogen release by earthworm’s metabolic products and dead tissues (Araujo et al., 2004).

The C/N ratio decreased during the composting and vermicomposting process. At the ambient stage, the C/N ratio of the composting materials was maintained at 24.18%. The microorganisms need about 25-40 times more carbon than nitrogen. Therefore it is important to provide carbon and nitrogen in appropriate proportions (Cabrera et al., 2005). As the composting process continued from mesophilic stage to thermophilic and cooling down stage the C/N ratio decreased to 19.38% (Table 1). During vermicomposting period, C/N ratio resulted in faster reduction from 17.92 to 10.15% as compared to compost without earthworms (Table 2). Guoxue et al. (2001) have recorded reduction in C/N ratio during composting process. The reduction in carbon and lowering of C/N ratio in the vermicomposting could be achieved either by the respiratory activity of earthworms and microorganisms or by increase in nitrogen by microbial mineralization of organic matter in combination with the addition of the worm’s nitrogeneous wastes through their excretion (Christry and Ramalingam, 2005). Research done by Reddy and Shantaram (2005) showed that the vermicomposting resulted in faster reduction of C/N ratio as compared to composts without earthworms.

Changes in moisture content

The moisture content at ambient stage was 65.52% and gradually reduced to 50% at the end of the cooling stage (Table 1). The vermicomposting process requires a moisture content of 70 to 90% (Tognetti et al., 2005); hence the moisture content was maintained between 70 to 75% (Table 2). In composting, the optimum moisture content varied from 50 to 70% (Crawford, 1985). The low percentage of moisture content reduces microbial biodegradation (Ahn et al., 2007) while at higher percentage, the water displaces much of the air in the pore of composting bed leading to anaerobic condition (Rynk, 2000). Therefore, the bulking agents such as sawdust, tree bank, straw, and dry leaves were added to maintain desirable moisture content in the composting process (Nakasaki and Ohtaki, 2002). Research has already shown that as the organic matter decomposes the moisture content decreases (Guest et al., 2001). The heat generated by biological metabolism and air flow increases the water evaporation in the bioreactor, consequently decreasing the moisture content. Decline in the moisture content percentage during the thermophilic phase of composting due to high evaporating rates has been recorded by Larney and Blackshow (2003).

WHC and EC

It was found that the water holding capacity (WHC) and electrical conductivity (EC) increases during the period of the composting and vermicomposting process (Tables 1 and 2). The increased water holding capacity may be due to aggregate nature of worm castings. Guoxue et al. (2001) found that the EC of compost increased slightly which may be due to the degradation of organic matter to release cations.

Changes in nutrient status

The vegetables waste was assessed for its nutrients sta-

Table 3. Concentration of nutrients during composting process of vegetable waste.

Treatment	Nutrients concentration (mg/100 g)						
	Ca	Na	Mg	Fe	Zn	Mn	Cu
Vegetables waste(raw)	1117.63	775.65	653.67	145.24	64.06	10.043	1.322
Ambient Stage	1357.57	1609.79	294.69	1014.56	401.72	25.76	3.640
Mesophilic Stage	1413.69	1466.09	309.52	888.36	358.45	26.75	3.668
Thermophilic stage	1789.48	759.63	315.60	848.34	356.84	41.99	4.961

Values are averages of three replicates.

Table 4. Chemical and nutrient status of vermicompost.

Parameters	Value
pH	6.8
Electrical conductivity (dS m^{-1})	10.55
Total C (%)	13.5
Total N (%)	1.33
Available P (%)	0.47
Sodium (mg /100 g)	354.68
Magnesium (mg /100 g)	832.48
Iron (mg /100 g)	746.26
Zinc (mg /100 g)	16.19
Manganese (mg /100 g)	53.86
Copper (mg /100 g)	5.16

Values are averages of three replicates,

tus. It has been found that the vegetable waste contained high concentration of nutrients such as Ca, Zn, Cu, Mg, Fe, and Mn. The nutrients status was also measured during the composting process, viz ambient mesophilic and thermophilic stages (Table 3). The vermicompost developed was characterized and high concentrations of various nutrients were found (Table 4). A similar increase in the status of nutrients had been obtained by Dickerson (1999). The concentration amount of micro nutrients viz, Fe, Mn, Zn, and Cu in vermicompost was found to be higher than ordinary ones by Reddy and Shantaram (2005). Thus, the final product i.e. vermicompost showed a considerable amount of mineral nutrients. Vermicompost has proven to have a higher level of plant available nutrients and therefore may enhance soil fertility (Paul, 2000).

Microorganism diversity monitoring

The dominant species of microorganism were isolated from vegetable waste, cow dung, soil, during composting, vermicomposting and investigated (Table 5). The microbes as represented at the ambient stage were *Pseudomonas* sp., *Streptomyces* sp. and *Bacillus* sp. The microbes found during the mesophilic were *Pseudomonas* sp., *Bacillus* sp., *Flavobacterium* sp., *Closteridium* sp. and S*treptomyces* sp. The thermophiles such as *Bacillus* sp., S*treptomyces* sp., *Thermoactinomyces* sp., *Thermonospora* sp. and *Micropolyspora* sp. were dominant during the thermophilic stage.

It was found that the *Bacillus* species were dominant during the entire process of composting. These observations are in conformity with those obtained by other authors (Fang and Wong, 2000; Gestel et al., 2003; Pedro et al., 2003). The decrease of microbial diversity in the composting mass could be due to the high temperature (Fang and Wong, 2000). The thermophilic bacteria played an important role in organic matter degradation during the thermophilic phase of the composting process (Zibilske, 1999). As the temperature declines, the actinomycetes and fungi become dominant during the composting (Jiarg et al., 2003) and vermicomposting process.

This study clearly demonstrated the potential of HBOB for composting followed by vermicomposting of market vegetable waste. The vermicompost developed has high nutrient values which serve as bio-fertilizer.

Conclusion

In the present study, the environmental friendly and low cost hydro-based operating strategy applied in the bioreactor for turning and aeration of the composting materials is suitable for inducing aerobic and potential decomposition especially during the early stage of composting (bedding). The thermophilic condition maintained for a period of one week in the developed bioreactor has a great influence on the inactivation of harmful pathogens in the bedding materials. The results of this work showed the simultaneous application of different approaches including physicochemical, biological and provides a more thorough description of the entire period of the composting and vermicomposting process. The vermincompost developed was found to have high value of nutrients.

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Table 5. Microbiological analysis during composting and vermicomposting.

Microaganisms	Vegetable waste	Cow dung	Soil	Ambient	Mesophilic	Thermophilic	Vermicompost
Acinetobacter	-	+	-	+	+	-	-
Alcaligenes	+	-	-	+	+	-	-
Bacillus sp.	-	+	+	+	+	+	+
Flavobacterium	-	-	-	-	+	-	-
Micrococcus	-	+	+	-	-	-	+
Nocardia	-	-	+	+	+	-	-
Penicillium	+	+	-	+	+	+	+
Pseudomonas sp.	+	+	+	+	+	+	+
Streptomyces sp.	-	-	-	+	+	+	+
Aspergillus	+	+	-	+	+	+	+
Rhizopus sp.	-	+	-	+	+	+	+
Closteridium sp.	-	-	+	-	+	-	-
Thermus sp.	-	-	-	-	-	+	-
Thermoactinomyces sp.	-	-	-	-	-	+	-
Thermomonospora sp.	-	-	-	-	-	+	-
Micropolyspora sp.	-	-	-	-	-	+	-
Staphylococci sp.	-	+	-	+	+	-	-
Salmonella Paratyphi B.	+	-	-	+	+	-	-

The plus sign (+) indicates present; the minus sign (-) means absent.

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