Effect of storage on the properties of vermicompost generated from paper waste: with focus on pre-drying and extent of sealing

M. Karthikeyan · S. Gajalakshmi · S. A. Abbasi

Abstract The beneficial effects of vermicast on soil fertility in general, and agriculture in particular, are widely recognized, but there are no reports on the effect of storage on vermicast quality. The present study is an attempt to cover this knowledge gap as it may assist in the formulation of guidelines for packaging and storing of vermicast in a manner that preserves the cast’s fertilizer value. Vermicast generated from paper waste was packed in airtight and partially sealed bags with and without pre-drying for 24 h. Changes in several physical, chemical, and biological properties of the castings were monitored for 3 months with weekly assessments. The results reveal that the beneficial properties of vermicast were the highest when it was fresh. There was deterioration on storage, which can be minimized if the castings are contained in airtight bags after pre-drying the casts.

Keywords Vermicast properties · Vermicast storage · Vermicast packing · Eudrilus eugeniae · Paper waste

Abbreviations
AUD Fresh vermicast stored in airtight bags
APD Vermicast pre-dried for 24 h stored in airtight bags
PUD Fresh vermicast stored in partially sealed bags
PPD Vermicast pre-dried for 24 h stored in partially sealed bags
WHC Water holding capacity
WFPS Water-filled porosity

Introduction

The vermicast that is deposited by the earthworms on the soil is known to fertilize the soil as well as influence its physical and chemical properties in a way that is beneficial to plant growth in particular and soil environment in general. Due to this realization, several studies have been conducted on the fate of vermicast, especially how the biological, chemical, and physical attributes of the vermicast change with time [1–11]. These studies have been on either vermicast generated from non-specific substrates in nature or from blends of soil and phytomass. The focus of the studies has been primarily on the stability of vermicast generated by anecic and endogeic (geophagous and...
geophytophagous) earthworm species as such casts are rich in soil and influence the stability of biogenic structures. Very few studies have been done on epigeic or phytophagous (‘humus feeder’) species. Moreover, when vermicast is deposited in nature, its fate is strongly influenced by (a) soil dwelling invertebrates—which colonize the vermicast and feed upon the organic matter it contains [5, 12]; (b) vegetation—which takes up the nutrients from the castings [13]; (c) soil microbes including autotrophic algae, nitrification bacteria or fungi—which are involved in fixation of atmospheric CO₂ [13]; (d) immobilization or mineralization of nutrients in the vermicast by soil microorganisms [14], and (e) environmental factors such as rain, flooding, or drought.

A few controlled studies have been reported on the change in the properties of vermicast upon aging [1, 6, 15–19]. These studies have primarily aimed to simulate the conditions which the biogenic structures experience in nature. For this, the casts were generated from either soil or blends of soil and phytomass and stored in soil/sand columns. It was seen that the soil particles present in the casts get chemically bound with organic matter, perhaps through chelation, which increases the stability of the casts. It also protects the organic matter content of the casts from decomposition [15, 20], as the organic matter that is attached to the minerals with strong chemical bonds is less accessible to microorganisms [21]. In addition, extracellular enzymes are protected from degradation or protolysis by the clay minerals contributed by the soil [22].

In contrast to the focus of the prior art summarized as above, the conditions associated with the storage of vermicast when it is produced by anthropogenically controlled vermicomposting and for the specific purpose of use as a fertilizer are very different. The concern here is to ensure that the vermicast retains as many and as much of the plant-friendly attributes as does fresh vermicast and the physical integrity of the cast is not of much significance. The only pre-existing studies on the effect of storage on vermicompost [3, 4], have been based on the use of 2-month-old press-mud as feed for earthworms, and assessment of the changes in major nutrients (N, P and K), microbial activity and enzyme activity of the vermicast that was generated. In these studies, the environmental conditions under which the casts have been exposed during the aging—either in vermireactors or in a controlled system—have not been defined. Also, the studies were done only at two stages—15th and 30th day of vermicast generation. Hence, no useful pointers can be drawn from these studies on the impact of storage.

The present study, which is perhaps the first of its kind, explores the changes in the physical, chemical and biological properties of vermicast that occur during storage with the objective of finding conditions that minimize the deterioration in the fertilizer value of the vermicast. The studies provide useful pointers on how best to store and package vermicast.

Materials and methods

Types of storage

The vermicompost used in the present work was generated from paper waste and the epigeic species, Eudrilus eugeniae. As paper waste is almost entirely cellulose, with only traces of elements other than C, H, and O, the feed was spiked with 9 % w/w of cow dung to provide NPK and other nutrients in adequate amounts. The vermicomposting was accomplished with a high-rate process recently developed by the author’s group [23]. The vermireactors were fabricated with aluminum sheets and each had a volume of 135 l (15 cm height with surface area 150 × 60 cm). The vermicast was harvested after 30 days. One part of it was stored in two types of packs: (a) airtight sealed transparent polyethylene bags of 20 µm thickness (AUD) and (b) partially sealed nylon mesh (0.3 mm) bags (PUD). Both types of bags were 25 cm long and 18 cm wide, each capable of holding half kg of vermicompost. Another part of the casts was pre-dried for 24 h at room temperature (29 ± 4 °C) and stored in both airtight sealed transparent polyethylene bags (APD) and partially sealed nylon mesh bags (PPD). In each set, 36 packs were utilized; overall 144 packs were studied. All storage was at room temperature (29 ± 4 °C) as this is the temperature at which vermicast is handled in the region where the authors work. Three packs of vermicast were taken once in a week for physical and biochemical analysis from each storage.

Analysis

Physical properties. To estimate bulk density, sample volume was measured with a graduated cylinder and its dry weight determined by oven drying [24].

- The particle density was determined by volumetric flask method [24]. The quotient value of weight of the sample and its volume which was measured through volume of water displaced by known amount of soil sample in the volumetric flask is reported as particle density.
- To measure the water holding capacity, the samples were filled in cylinders with a perforated base and immersed in water and drained. The quantity of water taken up by samples is determined by drying to constant mass at 105 °C [25].
- The total and water-filled porosity were calculated from the particle and bulk density values of the respective samples, using the following Eq. [26].
Total porosity \( (S_t) \) = \( 1 - \frac{D_b}{D_p} \) \hspace{1cm} (1)

Air filled porosity \( (F_a) \) = \( S_t - \theta_wD_b/D_w \) \hspace{1cm} (2)

Water filled porosity \( (WFPS) = \left( S_t - F_a \right)/S_t \) \hspace{1cm} (3)

where, \( D_b \) is the bulk density, \( D_p \) is the particle density, \( \theta_w \) is gravimetric water content, and \( D_w \) is the density of water at corresponding temperature.

- Electrical conductivity (EC) and pH were measured with suspensions of samples in water (1:2, w/v) [24] using EITM 611E EC meter and Digison TM digital pH meter 7007, respectively.

Chemical constituents. Total organic carbon \( (C_{org}) \) was determined following modified dichromate redox method [27]. External heating was applied during the oxidation process to quicken and complete oxidation of organic carbon in the sample.

- Dissolved organic carbon \( (C_{dis}) \) was extracted in 0.5 M K\(_2\)SO\(_4\) solution (1:10, w/v) and determined by the dichromate redox method [28].

- Total nitrogen \( (N_{tot}) \) was determined by modified Kjeldahl method [29] using Kel PlusTM semi-automated digester and distillation units. To include nitrate, nitrite, nitro and nitroso groups in the assay, a mixture of salicylic acid and sulfuric acid was used for digestion.

- Inorganic nitrogen was extracted with 2 M KCl solutions (1:10, w/v) followed by determination of ammonium \( (NH_4^+ - N) \) and nitrate \( (NO_3^- - N) \) in the suspensions by modified indophenol blue and Debardla’s alloy methods, respectively [30].

- Extractable potassium \( (K_{ext}) \), calcium \( (Ca_{ext}) \) and sodium \( (Na_{ext}) \) were determined using a flame photometer (ElicoTM CL378) after extraction with neutral 1 N ammonium acetate solution.

- Extractable phosphorus \( (P_{ext}) \) was determined according to the ammonium molybdate–ascorbic acid method [31] after extracting with Mehlich 3 extraction solution (1:25, w/v) [32].

- Mineral sulfur \( (SO_4^{2-} - S) \) was extracted with 0.0125 M CaCl\(_2\) solution (1:4, w/v), and determined by turbidimetric method described by Bashour and Sayegh [24].

Enzyme activity. Dehydrogenase enzyme activity (DHA) was estimated by determining the rate of reduction of iodonitro-tetrazolium chloride (9.88 mM) to iodonitro-tetrazolium formazan, after incubation at 40 °C for 2 h [33].

- β-Glucosidase (BGA), alkaline phosphatase (APA) and arylsulphatase (ASA) enzyme activities were assayed by incubation of samples with \( p \)-nitrophenyl glucoside (0.025 M), \( p \)-nitrophenyl phosphate (0.115 M) and \( p \)-nitrophenyl sulfate (25 mM) as described by Eivazi and Tabatabai [34, 35] and Tabatabai and Bremner [36] followed by the spectrophotometric determination of the released \( p \)-nitrophenol.

- Cellulase (CEA) activity was assayed by determination of the reducing sugars released after incubation of samples with carboxymethyl cellulose sodium salt (0.7 %) for 24 h at 50 °C, in a spectrophotometer at 690 nm [37].

- Urease (URA) activity was assessed by incubating samples with urea (200 mM) for 2 h at 37 °C, and measuring the \( NH_4^+ \) released in the hydrolysis reaction by steam distillation method using Kel PlusTM distillation unit [38].

- Microbial biomass carbon \( (C_{mic}) \) was determined by the chloroform fumigation–extraction method [28].

Processing of data

The experimental findings were statistically analyzed to assess whether different treatments exerted significant impact on the properties of vermicast over the course of the storage. Pearson correlation was used to estimate the degree of association between each of the vermicast properties studied and their influence over others. Statistical significance is recognized at \( P \) value ≤0.05. The SPSS windows 16 package (Softonic, Barcelona, Spain) was used throughout.

Results and discussion

Physical properties

The physical properties of vermicast were significantly affected by pre-drying and storage (Table 1). Pre-drying reduced the moisture content, WHC, total porosity, and WFPS, while it increased the bulk and particle densities of the cast (Figs. 1, 2). In the course of 12 weeks, the moisture content of the cast of PUD and PPD treatments was reduced by 69.4 ± 0.1 and 62.1 ± 0.6 %, and those of the AUD and APD by 5.7 ± 0.6 %, respectively. The bulk density increased in the PUD and PPD storage to the extent of 49.7 ± 0.8 and 29.1 ± 0.3 %, respectively. Structural compactness occurring due to water loss in drying may be the reason for the greater increase in the bulk density in the PUD and PPD storage. The WHC also reduced drastically due to this occurring in both PUD and PPD storage, in comparison to 14.1 ± 0.3 and 19.2 ± 1.2 %, respectively, in the AUD
and APD storage. A significant linear relationship ($P < 0.001$) was found between WHC and porosity of castings. At the end of the experiments, the cast in the PUD and PPD storage showed about 25% reduction in the total porosity and those in the AUD and APD about 6 and 12% reduction, respectively. Hydrophilic components of organic matter, such as polysaccharides, might also have influenced the WHC of castings [39].

The WFPS values increased to the extent of 22.4 ± 3.4 and 32.7 ± 5.8% in AUD and APD storage and decreased by 18.4 ± 2.0 and 8.5 ± 5.8% in the PUD and PPD storage, respectively. Reduction in structural pores during dehydration and decomposition of organic matter may be the reason for lower WFPS of the PUD and PPD storage [40, 41]. The changes in the WFPS of vermicast may have a distinct impact on the regulation of hydrological properties, gas diffusion, microbial colonization, nutrient mineralization etc. [42–44].

The particle density of cast showed an increasing trend in all the storage and the maximum of about 9% increase was observed in the PUD and PPD. The rate of increase in particle density of casts indicates the degree of decomposition of organic matter they contain [45, 46]. Increasing trend was also observed with EC, in which maximum increase of 33.5 ± 0.1 and 27.1 ± 2.6% was in AUD and APD storage and 15.5 ± 1.0 and 6.8 ± 3.4%, respectively, in PUD and PPD. The castings in all types of storage had pH close to neutral all the time (6.99–7.21); minor fluctuations occurred due to the production of organic acids and the release of CO2 during the microbial decomposition of organic matter [47, 48].

### Chemical properties

The chemical properties of cast were significantly influenced by pre-drying and storage ($P < 0.001$) (Table 2).
Fig. 2 Changes in the total porosity (a), water-filled porosity (b), pH (c) and EC (d) of undried and pre-dried castings stored in airtight sealed bags (AUD and APD, respectively) and undried and pre-dried castings stored in partially sealed bags (PUD and PPD, respectively), at different periods of time.

Pre-drying reduced the $C_{org}$, $C_{dis}$, $N_{tot}$, $NH_4^+$–$N$ and $P_{ext}$, and increased the $NO_3^–$–$N$, $K_{ext}$, $SO_4^{2–}$–$S$, $Ca_{ext}$ and $Na_{ext}$ content of the cast significantly. The PUD and PPD storage showed higher reduction in $C_{org}$, $C_{dis}$, $N_{tot}$, $P_{ext}$ and $K_{ext}$ than the AUD and APD storage (Figs. 3, 4). In the case of $C_{org}$, about 8 % reduction was observed with PUD and PPD storage and less than 1.5 % in AUD and APD. The higher reduction of $C_{org}$ in the PUD and PPD, which was maximum in the first 2 weeks, was probably due to rapid mineralization of C by aerobic microbes [49]. A few past studies on the aging of cast of anecics and endogeics have also reported high C mineralization in the initial period [7, 50, 51]. The reduction in WFPS with time may be influencing the extent of C mineralization as the days progressed because reduction in the water-filled pores during the storage might be curtailing the microbial access to the substrate [42, 52]. The cast in the PUD and PPD storage also showed a maximum reduction in $C_{dis}$ content: more than 90 % in comparison to 71.7 ± 0.6 and 88.0 ± 0.3 %, respectively, in the AUD and APD storage. Low C utilizing efficiency of anaerobic microbial community, which is expected to dominate in the AUD and APD storage could be the reason for the lesser reduction of $C_{dis}$ content in these types of storage [53, 54].

The $N_{tot}$ content of the cast reduced to the extent of 66.9 ± 0.5 and 54.6 ± 0.3 % in the PUD and PPD storage, respectively. There was about 30 % reduction in the $N_{tot}$ during the first week, probably due to intense ammonia volatilization. The high $NH_4^+$–$N$ content of fresh castings also supports this assumption. As the number of days progressed, the ammonium content of the casts in the PUD and PPD storage reduced up to 83 % due to the intense nitrification and ammonia volatilization from the existing ammonium pools. There was a concomitant increase in nitrate content of the cast over time: 72.2 ± 0.3 and 60.0 ± 2.0 % with the PUD and PPD storage, respectively, by the end of the study. Previous studies on cast aging have also reported rapid exhaustion of most of the ammonium present in the castings [8, 55, 56]. In contrast, the cast of AUD and APD storage showed reduction in both $NH_4^+$–$N$ and $NO_3^–$–$N$. Anoxic condition created in AUD and APD storage due to the airtight sealing might have impeded the nitrification process [57, 58], even as the slow reduction in the mineral nitrogen content of the casts may be due to microbial immobilization [2, 59].

In all cases, the $P_{ext}$ in cast increased during the initial week and further storage showed a steady decline till the end. The high availability of carbon and nitrogen in fresh cast would have increased the phosphorus demand and it probably enhanced phosphatase activity resulting in increased $P_{ext}$ during the initial week [3, 60]. As the number of days progressed, the $P_{ext}$ in the PUD and PPD storage reduced by 71.5 ± 0.3 and 73.9 ± 1.5 %. In AUD and APD, it was reduced by 68.0 ± 1.2 and 61.9 ± 1.6 %,
respectively. The \( K_{\text{ext}} \) in the casts also increased in the initial week, but further storage reduced it in all the treatments. This was particularly pronounced in the PUD and PPD when the reduction in \( K_{\text{ext}} \) was 16.2 \pm 2.1 and 27.5 \pm 1.6 \%; in comparison to 5.4 \pm 2.0 and 1.6 \pm 2.3 \% in AUD and APD, respectively. Similarly, the \( \text{SO}_4^{2-} \) content of the cast showed about 20 \% increase during the initial week, while further storage decreased it. The increase in the C/S ratio of the cast to above 600 that occurred during this period may be attributed to high immobilization of available sulfur \([61, 62]\). Throughout the experiment, the \( \text{Ca}_{\text{ext}} \) in the cast fluctuated in the AUD and APD storage. Increased solubility of organic carbon and increased competition between the cations for the negatively charged sites due to increased levels of Fe and Mn under reducing conditions may be the reasons for this fluctuation \([63, 64]\). The \( \text{Ca}_{\text{ext}} \) in cast of PUD and PPD storage steadily declined till the end, while \( \text{Na}_{\text{ext}} \) declined to the extent of 11.1 \pm 0.8 and 31.1 \pm 0.1 \% in the PUD and PPD storage and 19.0 \pm 0.6 and 23.2 \pm 0.5 \%, respectively, in AUD and APD.

Biochemical properties

Pre-drying of the casts had strong influence on the enzyme activities (Table 3). Except URA, the activities of all other enzymes assayed—DHA, CEA, BGA, APA and ASA—initially increased in the pre-dried cast before declining. The extent of this change varied with the type of storage (Fig. 5). As much as 82 and 77 \% increase in DHA was recorded in the first few weeks of AUD and APD storage, but further storage reduced the DHA activity to only 20.9 \pm 4.4 and 5.0 \pm 4.0 \%, respectively. In the case of PUD and PPD storage, DHA activity increased during the first week, and then declined to the extent of 89.0 \pm 0.3 and 97.6 \pm 0.2 \%, respectively. This trend may be due to enhanced growth of facultative anaerobic microorganisms caused by exhaustion of oxygen in these types of storage \([65, 66]\). Further storage reduced the DHA activity, possibly due to subsequent decline in the availability of nutrients.

The BGA activity of the casts increased to about 73 \% in the PUD and PPD storage by the second week. Then, there was a steady decline till it fell to 48.8 \pm 0.2 and 49.5 \pm 1.2 \%, respectively, at the end. Similar trend was observed with AUD and APD storage, in which there was about 67 \% increase in BGA activity during the second week, and 62.4 \pm 1.0 and 69.0 \pm 0.6 \% reduction by the twelfth week. The CEA activity of castings also increased with PUD, PPD and APD storage during the initial week, but then fell as much as sevenfold; those of AUD reduced ninefold. During the initial week, more than four times increase in APA activity was observed with the PUD and
PPD storage, and about twice with AUD and APD. Further storage reduced the APA activity in all the cases. In the AUD and APD storage, the reduction in APA activity was 40.8 ± 1.1 and 47.1 ± 1.2 % and in PUD and PPD storage it was 61.3 ± 1.0 and 71.4 ± 1.9 %, respectively.

During the first week, there was a slight increase in ASA activity with AUD and APD storage. In the case of PUD and PPD storage, the ASA activity increased up to third and fourth weeks, respectively. Further storage showed a drastic reduction in the ASA activity with all types of storage: 93.3 ± 1.2, 97.9 ± 0.2, and 99.4 ± 0.1 %, in AUD, APD, PUD and PPD, respectively. Fresh casts had the highest URA activity, which was reduced to 83.1 ± 0.3, 89.7 ± 0.3, 95.1 ± 0.3, and 94.8 ± 0.3 % in AUD, APD, PUD and PPD, respectively, at the end.

In summary, except URA, the activities of all other enzymes first rose in the initial weeks possibly due to high availability of nutrients and physical conditions favorable for aerobic microbial growth [67]. Subsequent decline in the nutrient content [68, 69], moisture content [70] and availability of oxygen [71, 72] with different types of storage may have contributed to the subsequent decline in the enzyme activities.

Pre-drying increased the C\textsubscript{mic} content of the casts significantly; it was 16.5 ± 0.7 % higher than the fresh ones (Fig. 6). During the first week, C\textsubscript{mic} increased to 20 % in the PUD and PPD storage, probably due to the high availability of nutrients which might have promoted high microbial activity during this period. Further storage led to reduction across the board: 70.0 ± 0.6, 78.2 ± 0.7, 93.8 ± 0.6, 96.3 ± 0.2 %, in AUD, APD, PUD, and PPD storage, respectively. Subsequent decline in the availability of nutrients and the moisture content in PUD and PPD storage may probably be the reason for the decline in C\textsubscript{mic}. In the case of AUD and APD, the reduction in C\textsubscript{mic} may be
attributed to the shift of aerobic microbial groups to anaerobes due to induced anoxic condition and which has very low C utilizing efficiency than the former [54].

Summary and conclusion

A 3-month-long study has been described on the effect of storage on the fertilizer value of vermicast. In what is arguably the first study of its kind, several physical, chemical, and biological attributes of the vermicast as stored with or without pre-drying, and with or without airtight containment, were assayed at 7-day intervals. The manner of storage was seen to influence the plant-friendly attributes of vermicast in a strong fashion. Airtight storage after pre-drying was the most beneficial, followed by airtight storage of the fresh, undried, vermicast. In partially sealed storage, there was significantly more rapid deterioration of the beneficial attributes than in airtight storage. Interestingly, whereas 24-h pre-drying before airtight storage was helpful in retaining the plant-friendly attributes of the vermicast for longer than fresh-airtight storage, pre-drying before partially sealed storage had the opposite effect. Apparently, partially sealed storage added to the water loss that had already occurred during the pre-drying, and brought the water content below a level that was needed to support biological activity within the vermicast matrix. This indicates that a certain level of water content is most appropriate for retaining the microbiological and enzyme activities of the vermicast; and the presence of water above or below that level hastens the cast’s aging. Further work should be aimed at determining the most beneficial water levels and how best to retain them.

Table 3  F values of repeated measures analysis of variance on the effect of extent of sealing and pre-treatment on biochemical properties of vermicast during the storage

| Treatment                        | Dehydrogenase activity | Cellulase activity | ß-Glucosidase activity | Urease activity | Alkaline phosphatase activity | Arylsulphatase activity | Microbial biomass carbon |
|----------------------------------|------------------------|--------------------|------------------------|----------------|-----------------------------|------------------------|-------------------------|
| Extend of sealing                | 54.07***               | 19.418.5***        | 1.393.6***             | 11.285.5***    | 4.538.0***                  | 2.654.1***             | 1.876.5***              |
| Pre-treatment                    | 98.894.9***            | 13.06***           | 12.427.8***            | 4.375.5***     | 5.307.3***                  | 65.483.6***            | 4.852.6***              |
| Extend of sealing X pre-treatment| 544.3***               | 11.275.7***        | 281.3***               | 3.477.9***     | 3.957.7***                  | 760.1***               | 4.894**                 |

* P < 0.05, ** P < 0.01, *** P < 0.001, n.s not significant
Fig. 5 Changes in the dehydrogenase (a), β-glucosidase (b), cellulase (c), urease (d), alkaline phosphatase (e) and arylsulphatase (f) enzymes activity of undried and pre-dried castings stored in airtight sealed bags (AUD and APD, respectively) and undried and pre-dried castings stored in partially sealed bags (PUD and PPD, respectively), at different periods of time.

Fig. 6 Changes in the microbial biomass carbon content of undried and pre-dried castings stored in airtight sealed bags (AUD and APD, respectively) and undried and pre-dried castings stored in partially sealed bags (PUD and PPD, respectively), at different periods of time.

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