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Compost Maturity Assessment Using Physicochemical, Solid-State Spectroscopy, and Plant Bioassay Analysis

D. Senthil Kumar,*,† P. Satheesh Kumar,‡ N. M. Rajendran,§ and G. Anbuganapathi†

†Department of Zoology, Kandaswami Kandar College, Paramathi Velur-638 182, Namakkal, Tamil Nadu, India

‡Department of Biological and Environmental Sciences, University of Messina, Messina-98166, Italy

§Department of Chemistry, Pondicherry University, Puducherry- 605 014, India

ABSTRACT: The vermicompost produced from flower waste inoculated with biofertilizers was subjected to compost maturity test: (i) physicochemical method (pH, OC, TN, C:N); (ii) solid state spectroscopic analysis (FTIR and ^{13}C CPMAS NMR); and (iii) plant bioassay (germination index). The pH of vermicompost was decreased toward neutral, C:N ratio < 20; reduced organic carbon with increased nitrogen indicates the compost attains maturity. The final vermicomposts result shows reduction of complex organic materials into simple minerals which indicates the maturity of the experimental vermicompost product than the control. The increased aliphatic portion incorporated with flower residues may be due to the synthesis of alkyl, O-alkyl, and COO groups by the microbes present in the gut of earthworm. Plant bioassays are considered the most conventional assessment of compost maturity analysis, and subsequently, it shows the effect of vermicompost maturity on the germination index of *Vigna mungo*.

KEYWORDS: *vermicompost, biofertilizers, compost maturity, FTIR, ^{13}C CPMAS NMR, germination index*

INTRODUCTION

The recycling of organic and inorganic waste minimizes the environmental hazards and adds a large amount of nutrients to intensive agricultural practices and also reduces the usage of chemical fertilizer.[1] The reuse of organic waste for soil quality improvement has received sound research attention. The recycling of bio-organic waste by the vermicomposting process decreases the difficulty of disposal of agricultural as well as industrial wastes. A high quality vermicompost has been produced by earthworms and its gut associated microflora within a short period of 3−6 months rather than thousands of years for the formation of humic acid (HA) by the natural process. Most of the decomposing organic waste products such as animal manure contain a huge amount of major and minor nutrients, enhancing the plant growth. Earthworm gut secretions contain an easily metabolizable compound that has been considered as one of the main sources for decomposition of waste materials by earthworms and microbial community.[2] Vermicompost is an excellent organic product with a low level of toxic content and holds large amounts of efficient nutrients to use as soil fertilizer without changing the environment.[3] A few studies have been conducted to test the suitability of vermicompost. Deportes et al.[4] stated that application of immature compost causes numerous ill-effects on soil in agricultural lands. However, it will also indicate the reduction of nitrogen (N) and polysaccharides, and arrest the utilization of nitrogen and phosphorus. Application of immature composts causes retardation of plant growth and damage to the plants by phytotoxicity and battle of oxygen, when they are applied inadequately as biodegradable organic matter.[5−7]

So far there is no evaluating method that has been universally accepted for composts maturity/stability. In most published research articles, the results were obtained from small scale research operations. Compost maturity/stability is an important factor that affects when the compost will used for agricultural purpose.[8−16] Vermicompost maturity and stability will be clearly proved by physicochemical and biological properties. More studies revealed the chemistry of decomposed organic matter (OM) content, and their functional group composition was used determine the vermicompost maturity. In the course of vermicomposting, changes occur in chemical structure, and transformation of compounds was analyzed by various methods: C/N ratio, humification process, and spectroscopical analysis (FTIR, ^{13}C NMR, and fluorescence spectroscopy).[11,15,17] FT-IR and ^{13}C NMR are the recent techniques to examine carbon composition of organic matter. These techniques are widely applied to analyze the compost maturity and characterization of organic matter.[18−20]

The ^{13}C CPMAS NMR technique was extensively used for evaluating vermicompost material in solid form, and the resonances in the ^{13}C CPMAS NMR were predicted by diverse carbon types: [21] (i) chemical shift range δ 0−50 ppm corresponded to alkyl C and fatty acids; (ii) chemical shift between 50 and 110 ppm was assigned to methoxy C in lignin, N-alkyl C in amino acids, O-alkyl C from carbohydrate, and di-O alkyl C from cellulose; (iii) chemical shift of phenolic C (δ = 150−160 ppm) fell in the range of the O-aromatic C chemical shift region (δ = 110−160 ppm); and (iv) chemical shift range δ 160−220 ppm includes C=O groups that are mainly a found in amino acids, ester, and quinones. Chen et al. [22] pointed out a reduction of carbohydrate content and increment of aromatic and carboxyl groups during decomposition of organic matter.

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Table 1. Physicochemical Parameters and GI of Vermicompost Samples[a]

samples	pH	TOC	TN	C:N ratio	GI
CO	7.4 ± 0.3	28.42 ± 1.22	1.9 ± 0.52	14.8 ± 2.0	58 ± 1.41
E1	6.9 ± 1.0	28.42 ± 4.38	2.03 ± 0.97	13.0 ± 1.0	100 ± 1.41
E2	7.2 ± 0.2	26.58 ± 0.68	2.29 ± 0.91	12.92 ± 1.0	115.5 ± 1.41
E3	6.86 ± 1.33	27.29 ± 3.19	2.2 ± 1.0	12.39 ± 0.05	110 ± 0.00
E4	7.2 ± 1.31	25.68 ± 0.91	2.25 ± 0.85	11.86 ± 4.1	112 ± 2.82
E5	7.06 ± 0.06	26.06 ± 1.0	2.29 ± 1.19	11.39 ± 0.1	142 ± 2.82
ANOVA	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.05$

[a]TOC, total organic carbon; TN, total nitrogen; GI, growth index.

In most research works, the conversion and stabilization of OM at the time of decomposition determines the compost maturity.[11,18,19,23,24] The aim of the present research is to evaluate the compost maturity of mixed flower waste vermicompost inoculated with biofertilizer by physicochemical method, spectroscopic techniques FTIR and ^{13}C CPMAS NMR, and plant bioassay (Germination Index).

■ MATERIALS AND METHODS

Flower waste and dry cow dung 1:1 w/w ratio were subjected to predecomposition for 30 days in plastic bins. The predecomposted substrate was mixed with biofertilizers (1g/kg of substrate) in plastic bins. Six feed mixes were prepared for the present study: control CO = substrate without earthworms; E1 = substrate with earthworms (*Eisenia fetida*); E2 = substrate + earthworms + *Azospirillum* (Azos); E3 = substrate + earthworms + phosphate bacterium (PB); E4 = substrate + earthworms + blue green algae (BGA); E5 = substrate + earthworms + *Rhizobium* (Rhizo).

After predecomposition, earthworms (*Eisenia fetida*) were inoculated (25 worms/bin). The moisture content was retained at 60−80%. The vermicomposting process was completed at the 80th day, and compost analysis was done at the end of composting.

Analysis of Total Carbon and Total Nitrogen. Total organic carbon (TOC) and total nitrogen (TN) content were analyzed by standard procedures.[25,26] The pH of vermicompost samples was determined using the procedure in ref 27.

Fourier-Transform Infrared Spectroscopy (FTIR) Analysis. Fourier-transform infrared spectroscopy (Perkin-Elmer 2000 spectrometer) was analyzed by taking 2 g of dried vermicompost sample along with 200 mg of KBr (spectroscopy grade), homogenized, and pelletized under vacuum. Readings were taken at a range 4000−400 cm^{-1} at a frequency of 0.5 cm/s.

^{13}C CPMAS NMR Spectroscopy Study. ^{13}C CPMAS NMR solid-state spectra were obtained via a DSX 300 Bruker spectrometer at the NMR research center, Indian Institute of Science (IISC), Bangalore, India. NMR operating conditions were as follows: SF frequency, 75.46 MHz; P3 contact chemical analysis time, 4.25 uses; Aq acquisition time, 0.29 s; D1 relaxation time, 5 s. In order to eliminate spinning side bands, spectra were recorded and several spectral editing techniques were used for assignments with ωr spinning rate, 10 kHz. The experimental procedures were similar to different studies on organic matter.[28,29]

Analysis of Plant Bioassay Analysis. A fresh extract of vermicompost samples, that is, vermiwash [10 mL] and distilled water [10 mL] was poured into a Petri dish with filter paper. Ten matured *Vigna mungo* seeds were placed on the filter paper and then incubated at 26 °C under dark cover for 4 days. The number of germinated seeds and their root radical lengths were measured. The germination index (GI) was determined by the formula:[30]

$$\text{GI} = \frac{\text{seed germination} \times \text{root length of the treatment}}{\text{seed germination} \times \text{root length of the control}} \times 100$$

Statistical analysis was performed using SPSS (version 7.5). One-way ANOVA and DMRT were analyzed with a homogeneous type of the data sets of different parameters in both control and treatments.

■ RESULTS AND DISCUSSION

Chemical Analysis of Vermicompost. In all the treatments (E1−E5), pH decreased over the control (CO) and was reduced toward neutrality. The reduction of pH was between the range of 6.8 and 7.2 which was statistically significant ($p < 0.01$). The decomposition of OM indicates the formation of humic acids and NH_4^+ ions.[31] Both of these components have the opposite effect on the pH. Carboxylic, phenolic, and humic acids reduce the pH value, and at the same time ammonium ions accelerate the pH value of the vermicompost, resulting toward neutral pH value.[32] Neutral pH in the vermicompost should be a vital factor to keep a hold on the nitrogen to encourage the nutrient accessibility of plants.[33]

The initial nitrogen level in the control was 1.9%, which was boosted to 2.29% in the final product (Table 1). A maximum TN level was recorded in E5 and E2 followed by E4, E3, and E1, which was statistically significant ($p < 0.001$). Earthworms in addition boost the N content by the accumulation of their excretory products in the compost.[34] Kaushik and Garg[35] performed inoculation of nitrogen fixing bacteria during the composting, and observed and increase in the nitrogen content of the final product. The total organic carbon (TOC) value in the initial stage was higher (36%) which was reduced toward the end of the 80th day of composting by 25%, thus representing the occurrence of extended mineralization. The results of organic carbon showed as significant at $p < 0.001$. The declines were observed in the C/N ratio of the initial value (15%) in the control and moreover at the end of the composting (11.5%) in E5, which indicates advanced organic carbon decay and the reaching of a stable level. The C/N ratio of flower waste enriched with biofertilizer was significant at $p < 0.001$. This present study observed a significant reduction of TOC, C/N ratio, and pH in vermicompost in all the experimental mixtures, similar to earlier described studies.[36,37]

FTIR. The assignment of absorption bands in FTIR spectra of vermicompost samples was done based on methods Williams and Fleming,[38] and results are presented in Table 2. The FTIR spectra of vermicompost samples showed (Figure 1) a broad band at $\nu \sim 3450$ cm^{-1} corresponding to O−H stretching frequencies of alcohol and phenol. The appearance of two distinct weak bands at ν 2925 and 2850 cm^{-1} suggested C−H stretching vibration of alkyls, and weak bands in the range of ν 2358−2360 cm^{-1} were observed for phosphonates. A small shoulder associated to nonconjugated carboxylic C═O stretching at ν 1791−1733 cm^{-1} was observed in control compost CO and E4 which disappeared when the pH reached

Table 2. Assignment of Absorption Bands in FTIR spectra of Vermicompost Samples

band position (cm^{-1})	assignments
3300−3500	O−H vibrations of hydroxyl groups of phenol, alcohols, and carboxyl functions and N−H vibrations from amides and amines
2920−2960	symmetric stretching CH_3 and CH_2 of aliphatic chains
2840−2855	asymmetric C−H stretching CH_3 and CH_2 of aliphatic chains
2300−2400	phosphonates
2200−2700	carbonyl absorption band in addition to an ammonium band
2000−2573	C═N stretching in secondary amides
1720−1740	C═O aldehyde
1600−1650	C═O amide I, aromatics
1380−1385	N−O stretch nitrate in solid waste samples
1080−1095	C−O−C polysaccharides
790−1030	Si−O−Si stretch of clay minerals
750−850	C−O carbonate

the neutrality. Hence, the pH affects only carboxylic acid groups that are involved in the acid−base reaction; a similar observation was reported in the literature.[39,40] The bands at $\nu \sim 1640-1650$ cm^{-1} lignin were also observed.

Nakamoto reported that a peak appearing between $\nu \sim 1361$ and 1388 cm^{-1} in the IR spectrum confirmed the complex formation of organic matter with heavy metal[41] and also this peak is not pH dependent.[42] Similarly, vermicompost samples exhibited a band in the IR spectra at $\nu \sim 1368$ cm^{-1} that may be attributed to the C−N stretch of amine groups in which the organic ligand involved coordination with heavy metal. Nitrates N−O stretching frequencies were observed in the range of $\nu \sim 1386-1384$ cm^{-1}. A slight broad band around 1095−1030 cm^{-1} relates to C−O−C groups in polysaccharides. The sharp band appearing at $\nu \sim 785-800$ cm^{-1} was related to C−O stretching of carbonate and silica as given in the literature.[39,43,44]

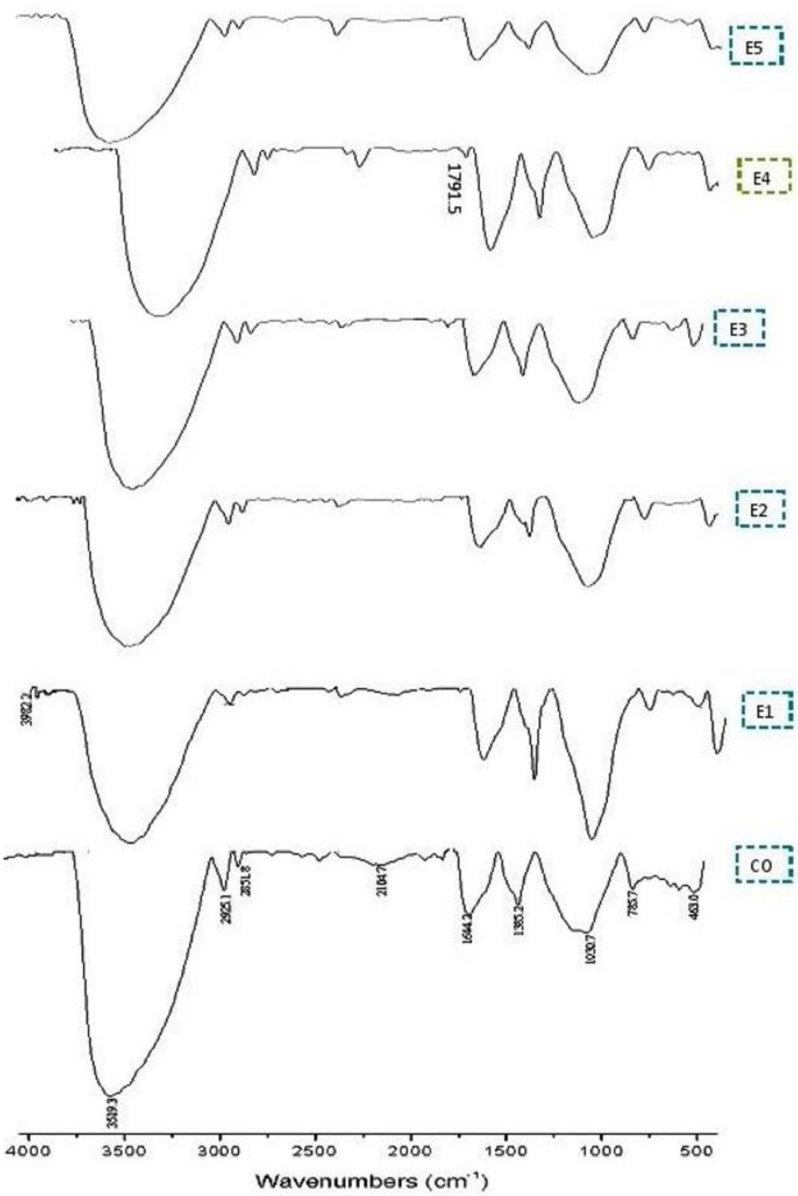

Figure 1. FTIR analysis of final control and experimental flower waste vermicomposts.

Table 3. Relative Distribution (%) of Signal Area over Chemical Shift Regions (ppm) in ^{13}C CPMAS NMR Spectra of Vermicompost Samples[a]

samples	alkyl C (0−50)	alkyl C−O 50−110	aromatic C (110−160)	carbonyl C (160−190)	ketonic C (190−230)	aliphaticiy (%)	aromaticity (%)	HI/HB
CO	6.35	3.74	1.42	5.705	0.86	87.66	12.33	0.8226
E1	4.65	10.94	1.71	3.24	0.53	90.12	9.88	0.4485
E2	7.17	3.64	1.67	3.74	0.44	87.77	12.22	1.1978
E3	6.575	3.575	1.38	4.75	0.14	88.03	11.97	0.9556
E4	2.34	7.46	1.10	3.64	0.325	89.91	10.09	0.3099
E5	3.0	10.73	0.415	4.465	0.18	97.07	2.93	0.2247

[a]Aliphaticity (%) = [aliphatic C peak area (0−110 ppm)]100/[total peak area (0−160 ppm)]. Aromaticity (%) = [aromatic C peak area (110−160 ppm)]100/[total peak area (0−160 ppm)]. Hydrophilic carbons/hydrophobic carbons = [(50−110) + (160−230)]/[(0−50) + (100−160)].

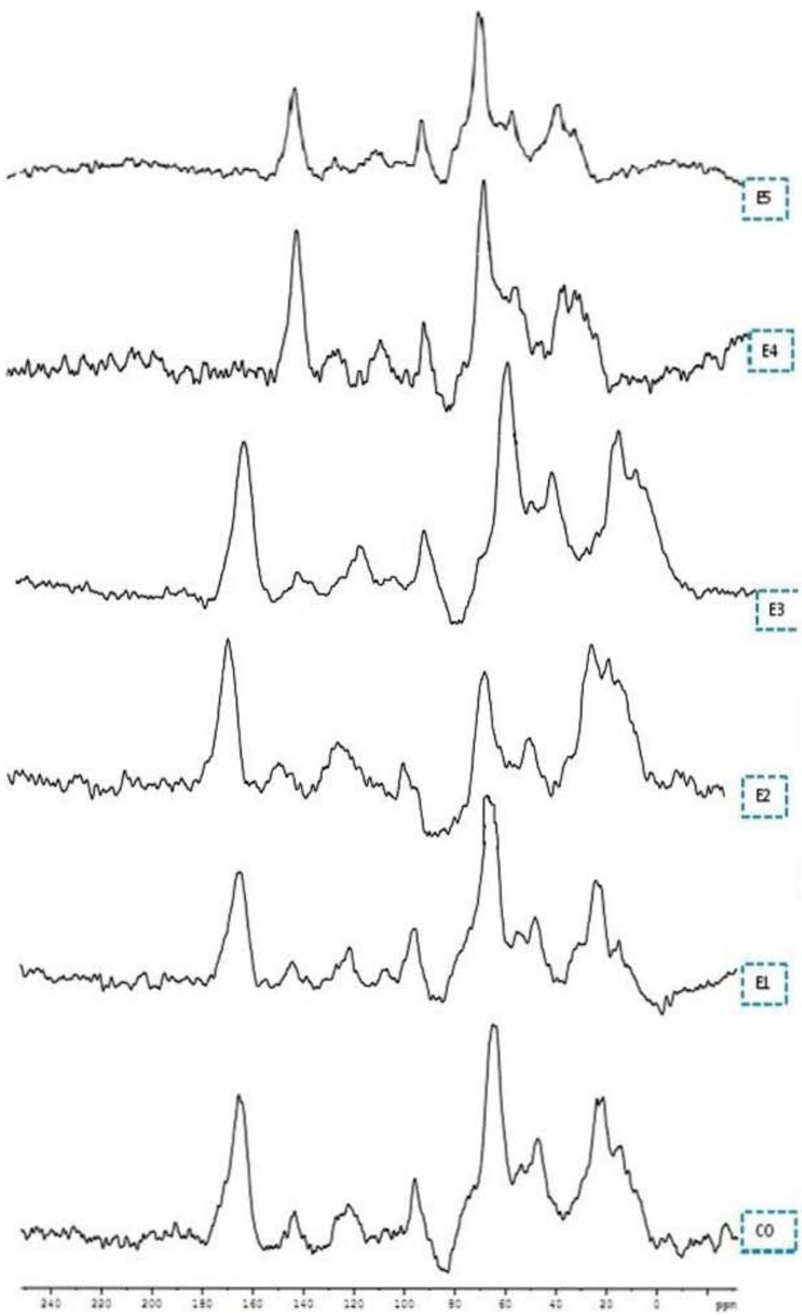

Figure 2. NMR analysis of final control and experimental flower waste vermicomposts.

^{13}C CPMAS NMR. NMR data for all the vermicomposting samples are detailed in Table 3, and spectra are shown in Figure 2. The presence of the alkyl groups (terminal methyl) and methylene groups (aliphatic rings and chain) in ^{13}C NMR spectra are evident by the appearance of two distinct resonances around δ 21 and 31 ppm, respectively. It has been reported in the literature that the resonances between δ 46 and 67 ppm can be attributed to methoxy C from lignin residues and N-alkyl C of protein residues.[45] Based on this study, the more intense peak at δ 56 ppm could be assigned to methoxy groups in lignin in the form of sinapyl and phenolmethoxyl of coniferyl moieties and further methoxy C available in hemicellulose (glucoronic acid in xylan). The chemical shift region between δ 60 and 110 ppm corresponds to polysaccharides and proteins. A similar observation (polysaccharide chains) is reported in the case of plant woody tissues.[46] Signals appearing around 75 ppm were due to C4 of cellulose, while hemicellulose signals also fall within the cellulose peak range. The chemical shift range at δ 114−122 ppm corresponds to aromatic C. The small peak at δ 141 and a dominant peak at δ 143 ppm were assigned to methoxy attached aromatic C. The long chain polysaccharides were easily digested with the help of gut microbial colonies, resulting in change of the structure of lignin into new polysaccharides and humins. The polysaccharides in the final vermicomposted materials were transformed into rich aliphatic C. From the ^{13}C NMR spectra, we concluded that aliphatic C content is greater than aromatic C content, and similar results were reported.[39,47,48] The higher aliphatic content incorporated with decomposition of flower residues may be due to the production of alkyl, O-alkyl, and carboxyl carbon by the microbial decomposer community in the gut of the earthworm.[45]

Plant Bioassay Analysis: Seed Germination. The plant germination was mainly used to evaluate the vermicompost maturity based on a seed germination index (GI) and initial rate of plant growth using a liquid extracted from the compost.[30] The extracts of compost at different stages of decomposition were incubated 24 h at 26 °C. All the vermicompost samples yielded high germination rates (80%) in contrast with the germination rate of control compost C (50%). The results of germination index (GI) of *V. mungo* were significant at $p < 0.05$ (Table 1). The neutral pH value of vermicompost samples has no negative impact on the seed germination of *V. mungo*. Zucconi et al.[30] reported the phytotoxicity of composts in terms of a cross germination bioassay by using *Lepidium sativum*. A number of earlier investigations have been attempted to determine the compost maturity and reported that vermicompost enhances seed germination.[49,50]

This present study of physicochemical and solid state spectroscopic (FTIR and ^{13}C NMR) analysis revealed that biofertilizer mixed flower waste vermicompost consists of a high degree of condensed aliphatic structures. Highest reduction of C:N ratio in decomposing OM is evident of the highest degree of compost stability by abolition of higher nitrogen content in the substrates. The high nitrogen content of the compost material agrees with the presence of FTIR vibrational frequencies resulting in secondary amide groups, suggesting the existence of peptide chains in the compost by way of protein degradation processes. ^{13}C CPMAS NMR study is a most reliable method to predict the organic matter transformation, The spectra exhibited an increased percentage of aliphatic compounds and lesser aromatic compound followed by decreased carboxyl group content, extensively a rapid degradation of polysaccharides as well as cellulose during the decomposition process.

These consequences are sustained by the fall of aromatic structures and the rise of O-alkyl and carbonyl carbon, a wide mineralization of OM during the vermicomposting process. The higher germination index (GI) reflects no phytotoxic compounds present in the biofertilizer enriched vermicompost samples. FTIR and ^{13}C CPMAS NMR are confirmed to screen the maturity of vermicompost during the composting process. It was concluded that biofertilizer enriched flower waste vermicompost fulfills the compost maturity test and is ready for agricultural and aquaculture applications.

AUTHOR INFORMATION

Corresponding Author

*E-mail: drskumar1968@gmail.com.

Notes

The authors declare no competing financial interest.

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