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ORIGINAL ARTICLE

Biological indicators for assessing the maturity of the vermicomposted products of citronella bagasse and paper mill sludge mixture

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Abstract

Maturity of vermicomposted end products obtained from citronella bagasse (CB) and a mixture of citronella bagasse and paper mill sludge (CB + PMS) was determined employing the epigeic earthworm species *Eisenia fetida*. Two of the primary biological indicators—enzyme assay and germination index—were used for evaluation of vermicompost maturity. The enzyme assay includes the changes in dehydrogenase and urease activities in the final products. The test for germination index was performed on two economically important plant species *Abelmoschus esculentus* and *Solanum melongena*. The results revealed a significant increase in dehydrogenase and urease activities in the vermicompost samples over the raw materials and traditional compost. The enhancement was recorded 215.63–225.63% in dehydrogenase and 149.89–249.31% for urease in the end products obtained after 45 days of experimental trials. Further, the higher values of germination index in the vermicompost treatments as against the control confirm the suitability and stability of the vermicomposted final products.

Keywords Stabilization · Vermicompost · Germination index · Dehydrogenase · Urease

1 Introduction

Huge quantities of organic waste are generated annually in the form of bagasse and sludge from citronella oil and paper mill industries, respectively. According to a recent estimate, India generates more than six million tons of aromatic spent biomass per year [1] as against the humongous 20,000 million tons of worldwide production [2]. The intensity of pollutants generated from the paper mill can be understood by the fact that it generates 0.3–1.0 m^3 of primary sludge for per ton paper produced [3]. Among the several strategies employed in past to mitigate the problems of solid waste, vermicomposting has been recognized as an eco-friendly, cost-effective and efficient practice. The vermicomposting has been proven as an efficient approach for bio management of citronella bagasse and paper mill sludge mixture [4]. However, the primary necessities for vermicomposted products are its safe agronomic use or land applications [5]; therefore, further investigation is needed to understand the maturity/stability of the end products by employing reliable indicators.

Besides the conventional methods, the modern techniques that are primarily based on instrumental analysis are being

Statement of Novelty The safe agronomic use and land applications are very important for any vermicompost products. Hence, proper evaluation of maturity or stabilization of vermicomposting end products is very essential. The physicochemical parameters that are used for understanding vermicomposting maturity have shown several limitations, and therefore, application of biological indicators has been preferred now a day. Thus, in the present study, efficacy of biological indicators for understanding the stabilization/maturity of the vermicomposting products of citronella bagasse and paper mill sludge was investigated. The activity of two very important enzymes viz. dehydrogenase and urease enzyme was found significantly higher in the vermicompost as compared with initial raw materials and compost which is accompanied by germination index value of more than 80% in the vermicompost and it is an indication of absence of any phytotoxic compound in the final products.
Biological evaluations have established vermicomposting end products as mature, beneficial and suitable potting media for agricultural and horticultural practices. This is the overall significance of this research.

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used heavily to understand the stabilization/maturity of the vermicomposted end products [6]. The physical characteristics such as colour, odour and temperature provide an idea about the decomposition stage but cannot give any information regarding the maturity of the compost/vermicompost [7]. The physicochemical parameters, more particularly changes in pH, electrical conductivity (EC), organic C, total N, C/N ratio, humification index (HI) and ash levels, are still used as reliable indicators for confirming the stability of the vermicompost materials. Further, the instrumental analyses such as Fourier transform infrared (FT-IR) spectroscopy, scanning electron microscopy (SEM), ultraviolet-visible (UV-Vis) spectroscopy, atomic absorption spectroscopy (AAS) and thermogravimetry (TG) are also in use for determination of the maturity of vermicompost samples [8, 9]. However, all these indices have several limitations. For example, C/N ratio which is used frequently for measuring the maturity of the composting/vermicomposting process showed wide fluctuation in values ranging from 12 to 25 and optimum value is even source dependent [5]. Similarly, instrumental analysis such as SEM and FTIR requires prior knowledge of the samples, frequent calibrations; besides, it must also be free from any possible electric or magnetic interference during analysis for better interpretations [6]. Therefore, the need and importance of biological indicators such as germination index (GI) and enzyme assay for verification of maturity of vermicompost steps in. Biological indicators, primarily germination index, hold a significant role in the determination of vermicompost maturity as it is directly associated with maturity and phytotoxicity of the compost for agricultural applications [10]. Use of biological techniques such as enzymatic analysis for vermicompost stability evaluation becomes very popular as it is precise, easy, relatively inexpensive [5] and more reliable prior to field applications.

Various researchers have already exploited the potential of biological indicators in the assessment of the maturity of vermicompost. The quantification of urease and dehydrogenase has been a well-established biological indicators for assessing the stability of the vermicomposting process [5, 11]. A very recent study on vermicomposting of coir industry waste revealed that the final products with dehydrogenase and urease activity of 46 μg TPF g^{-1} dwh^{-1} and 45 μg NH4 g^{-1} h^{-1}, respectively, yield matured vermicompost with no potential phytotoxicity with GI percentage climbing up to 128% [12]. It has also been suggested that urease and dehydrogenase are released by microbes during the vermicomposting process and play a crucial role in the biotransformation of the waste materials of different origin [13–15]. Moreover, researchers have successfully tested the maturity of vermicompost through germination assay and recommended it as a potential biological indicator for assessment of the maturity of the vermicomposted products [14, 16, 17]. For example, vermicomposted products of urban green waste showed higher germination index values which were even proven to be crucial for the phytotoxicity analysis [18]. Nevertheless, despite the amount of work done in this field, reports regarding determination of maturity of vermicompost of industrial waste mixture through biological indices are still scanty. Therefore, the present study was conducted to understand the role of urease and dehydrogenase enzymes in the stabilization of vermicompost end products of citronella bagasse and paper mill sludge mixture. Besides, the maturity of the vermicompost products were also evaluated by seed germination studies to know the further agricultural application potential.

2 Materials and methods

2.1 Collection of raw materials, experimental seeds and Eisenia fetida

The citronella bagasse (CB) was taken from citronella oil industry situated at Rajapara, Assam, India. Similarly, paper mill sludge (PMS) that results after the final step of the paper production process was collected from Nagaon Paper Mill, Jagirod, Assam, India. Both the raw materials were air-dried and processed well for use in the experiment. The *Eisenia fetida* was procured from the in-house vermicomposting unit of Institute of Advanced Study in Science and Technology (IASST), Guwahati, Assam, India. The earthworm's stock culture was maintained in the laboratory for use in the experiments. The seeds of *Abelmoschus esculentus* and *Solanum melongena* were collected from the nearby daily market.

2.2 Experimental set up

The experiment was carried out in pots of 2-L capacities (depth 18.5 cm, diameter 17 cm) for 45 days engaging two vermicomposting treatments. In the first treatment, citronella bagasse (CB) was used alone as raw material whereas citronella bagasse (CB) and paper mill sludge (PMS) were mixed thoroughly in 3:2 ratio (CB = 150 g, PMS = 100 g) in case of the second treatment. There was 15 days of predecomposition of the raw materials in respective pots to create a conducive environment for survival, growth and development of earthworms [19]. At the bottom of each pot, a bed for earthworms was prepared by adding pieces of small bricks and stones, soil and 15 g of semi decomposed cow dung. Twenty individuals of *Eisenia fetida* of about 20 days old were introduced to the experimental pots from the stock culture. The experiment was conducted in the ambient condition in the laboratory where the temperature ranges 25–30 °C during the entire period of the experiment. The moisture level in the pots was maintained at 70 ± 10% by sprinkling distilled water [20]. A similar setup designated as control was

maintained without adding earthworms. In each of the cases, 250 g of raw materials was taken maintaining three replicas for statistical comparison of the results. The experimental pots were covered with newspaper and tiny holes were made over it for good aeration [4].

2.3 Quantification of dehydrogenase and urease

Dehydrogenase activity of the samples was analysed by employing the method of Garcia et al. [19], with slight modifications. To analyse the dehydrogenase activity, 0.5 g of sample was incubated at 25 °C for 24 h with 0.2 mL of 0.4% 2-iodophenyl-3 p-nitrophenyl-5 tetrazolium chloride (INT) as a substrate. Production of iodonitrotetrazolium formazan (INTF) as a result of reduction of INT was extracted by employing a mixture of acetone/tetrachloroethene (3:2) and quantified spectrophotometrically at 250 nm and results were expressed as μg INTF g^{-1} h^{-1}. Assays without INT were used as a control treatment for this test.

The method proposed by Hoffmann and Teicher [20] was used for the study of urease activity in the samples. For analysis of urease activity, 0.25 mL toluene, 0.75 mL citrate buffer (pH, 6.7) and 1 mL of 10% urea substrate solution were added to the 1-g sample and the samples were incubated for 3 h at 37 °C. The formation of ammonium was determined spectrophotometrically at 578 nm and results were expressed as μg NH_4^+ g^{-1} h^{-1} dry sample. A control without adding any urea to each samples was also analysed for comparison of the results.

2.4 Phytotoxicity analysis

The phytotoxicity test was conducted as per the method suggested by Zucconi [21] with little modifications. Vermicompost extracts were prepared by adding 1 g vermicompost (dry weight basis) to 10 mL distilled water. The slurry was then filtered by using Whatman no. 1 filter paper. The seeds (10 each from both the experimental plant) were then placed on a Petri dish with a filter paper moistened with vermicompost (CB vermicompost and CB + PMS vermicompost) extracts over it. Another set-up was maintained by adding extracts from the natural compost. A control set-up was prepared with 10 mL of distilled water only. In each case, three replicas were maintained for comparison of the results. The moisture contents in the filter paper were maintained by sprinkling distilled water throughout the study period. The final germination counts were recorded on the eighth day of the experiment. The results are expressed as an index (germination index), GI [GI = (% G × % L)/100] combining relative germination (% G) and relative root elongation (% L), and compared with a distilled water control.

$$\text{Relative germination (\%G)} = \frac{\text{Number of seeds germinated}}{\text{Number of seeds kept for germination}} \times 100$$

$$\text{Relative root elongation (\%L)} = \frac{\text{Root elongation in the treatment}}{\text{Root elongation in control}} \times 100$$

2.5 Statistical analysis

Each treatment of the vermicomposting experiment was considered as an independent variable. Variations in the investigated parameters such as dehydrogenase, urease, and seed germination assay were subjected to analysis of variance, Duncan's test and Pearson's correlation coefficient ($P < 0.01$ and $P < 0.5$) using SPSS (Version 2018).

3 Results and discussions

3.1 Enzymatic study

The results of dehydrogenase enzyme activities have been presented graphically in Fig. 1. The results showed 13.87% and 225.11% enhancement in dehydrogenase activities in compost and vermicompost samples, respectively, as against the initial substrate, i.e. CB. Similarly, there was 11.24% and 215.63% increase in dehydrogenase activities in the compost and vermicompost samples, respectively, over the initial values of substrate mixture, i.e. CB + PMS. The variation in enhancement in dehydrogenase activities in the compost and vermicompost samples of CB and CB + PMS can be attributed to the differences in microbial/earthworm activities between the two types of feeding materials. This study falls in the line with previous workers [13], where they have reported about the similar pattern of increase in dehydrogenase activities in the vermicomposted samples of industrial waste. Dehydrogenase is an indicator of microbial activity of the sample because it occurs intracellularly in all the living microbial cells and has a direct influence on the overall microbial

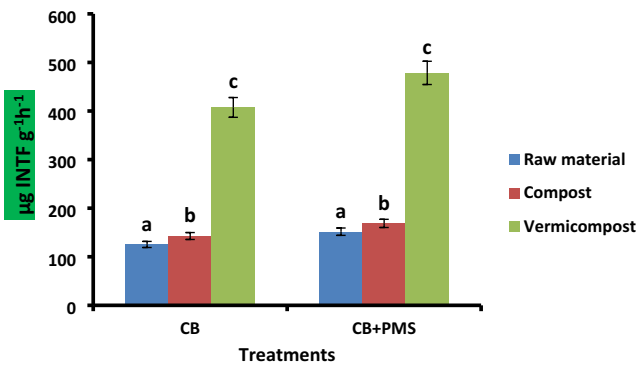

Fig. 1 Dehydrogenase enzyme activity of raw materials, compost and vermicompost samples. Values are mean, $n = 3$, bars indicate SD. Significant differences are indicated by different letters (CB = citronella bagasse; PMS = paper mill sludge)

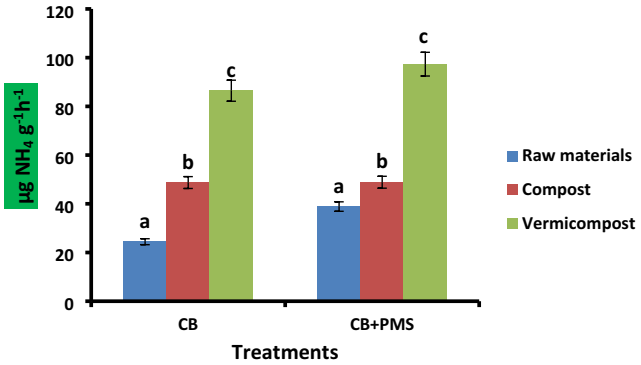

Fig. 2 Urease enzyme activity of raw materials, compost and vermicompost samples. Values are mean, $n = 3$, bars indicate SD. Significant differences are indicated by different letters (CB = citronella bagasse; PMS = paper mill sludge)

biomass of the treatment [21]. Dehydrogenase activity can also be related to the metabolic state of the microbial population of soil and maturity of vermicomposted products [15, 22]. Here, it is inferred that the dehydrogenase enzyme activity may increase due to the continuous accumulation of extracellular enzymes in humic substances which becomes stabilized and resistant to physical and microbial degradation resulting in a more stable or matured product.

The results of urease activities of the raw material, compost and vermicompost have been presented in Fig. 2. Urease catalyses the transformation of urea present in the substrate into carbon dioxide and ammonia [23]. In the treatment where CB was used as raw material, the urease activity was found to be increased by 35.93% and 249.31% in compost and vermicompost samples, respectively, whereas in the treatment where CB + PMS were used as raw material, the increase in urease activities was found to be 25.68% in the compost and 149.89% in the vermicompost samples. This study falls in line with the previous studies where an increase in urease activities was reported as 147 µg NH_4 g^{-1} h^{-1} in the matured vermicompost [14, 24, 25]. Since the urease enzyme is involved in the hydrolysis of proteinaceous substances to ammonia which as a consequence contributes nitrogen to the substrates resulting in a more matured end products known as vermicompost [22]. The increase in maturity of end products during vermicomposting can be correlated with an increase in the overall microbial population. Microbes enter in the earthworm's gut, consume the nitrogenous compounds of mucus and increases the microbial activities, which in turn enables them to contribute enzymes in the digestive process of earthworms [26]. This may be the reason for higher enzyme activities in vermicompost as compared with raw materials.

3.2 Phytotoxicity of vermicompost

Germination percentage was calculated prior to the calculation of germination index (GI) for obtaining a clear picture about the effect of vermicompost in the germination abilities of the experimental seeds and the values are presented in Fig. 3. The values of germination index (GI) were calculated for all the treatments and presented in Table 1. The GI values for the control were recorded as 80.24 ± 0.26 and 86.39 ± 0.92 in the seeds of *Abelmoschus esculentus* and *Solanum melongena*, respectively. The GI values for seeds of *Abelmoschus esculentus* were recorded as 90.11 ± 2.41 and 154 ± 4.87, respectively, in the water extract of CB compost and CB vermicompost samples. Similarly, for seeds of *Solanum melongena*, the GI values were found to be 94.36 ± 3.92 in CB compost and 161 ± 3.98 CB vermicompost water extract treatment. On the other hand, in case of CB + PMS compost and vermicompost, the values for GI of *Abelmoschus esculentus* seeds were recorded as 97.49 ± 0.69 and 195 ± 5.25, respectively, as against the 96.21 ± 1.67 and 191 ± 4.24 as obtained in the seeds of *Solanum melongena.*

The results from this study fit perfectly with the previous findings where it was suggested that GI values below 50% indicate high phytotoxicity; values between 50 and 80% indicate moderate phytotoxicity; values above 80% indicate the absence of phytotoxicity, and when the index exceeds 100%, the compost can be considered as phytonutrient or phytostimulant [17, 27]. The germination index of 130% as obtained in the vermicomposted samples of tannery waste has been reported as matured vermicompost [28, 29]. The mature vermicompost with more than 80% GI value can be regarded as a nontoxic manure for practical application in the

Table 1 Germination index (GI) of the seeds of *Abelmoschus esculentus* and *Solanum melongena* in control and extracts of compost and vermicompost samples

Seeds	Control	CB compost	CB + PMS compost	CB vermicompost	CB + PMS vermicompost
A. esculentus	80.24 ± 0.26a	90.11 ± 2.41c	97.49 ± 0.69e	154 ± 4.87d	195 ± 5.25f
S. melongena	86.39 ± 0.92b	94.36 ± 3.92 g	96.21 ± 1.67 h	161 ± 3.98 k	191 ± 4.24 m

Mean value ± SD, $n = 3$; different letters in the same row indicates statistically different values (ANOVA, $P < 0.05$)

CB citronella bagasse, *PMS* paper mill sludge

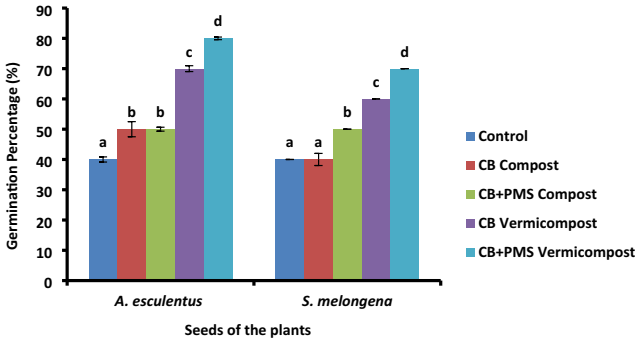

Fig. 3 Germination percentage of the seeds of *Abelmoschus esculentus* and *Solanum melongena*. Values are mean, $n = 3$, bars indicate SD. Significant differences are indicated by different letters (CB = citronella bagasse; PMS = paper mill sludge)

horticultural and agricultural practices [30]. The statistically significant ($P < 0.01$) GI values of vermicompost as compared with the initial raw materials and compost could be a result of conversion of ammonical nitrogen into available forms and rate of mineralization of organic matter along with the rate of degradation of organic acids [31]. Moreover, the reduction of phenolic compounds as a result of joint action of microbes and earthworms in the vermicompost can be a major factor for lowering the phytotoxicity of the extracts [12]. The stability of vermicompost obtained from CB + PMS was already examined in our previous study through physico-chemical parameters such as pH, electrical conductivity, ash content, total organic carbon, available nitrogen, C/N ratio, humification index, total phosphorus, total potassium and others [4]. Although physico-chemical parameters can provide a clear indication of the stability of the vermicomposted end products, the maturity of vermicompost can only be confirmed via a phytotoxicity test involving germination assay [32]. Here, the higher values of germination index can be attributed to the fact that the earthworms provide a conductive environment in the vermicompost samples leading to better mineralization and maturity of the vermicompost. The results of this study provide a good indication that vermicomposting can be a meaningful and sustainable technology for the elimination of phytotoxic effects of industrial organic waste and can be useful in organic farming of economically important crops.

3.3 Correlation studies

The correlation studies for dehydrogenase and urease activities and germination index have been carried out and results are presented in the Table 2. The correlations were represented by the upper triangle for CB and lower triangle for CB + PMS treatment. All the values exhibited a statistically significant positive correlation among each other. The value of correlation coefficient tends to turn around 1 which signifies that the maturity of vermicompost is significantly higher as compared with the initial raw materials and compost. These positive results indicate that the biological indices used in this study can be successfully implemented for studying the end product of a vermicomposting process.

4 Conclusion

Vermicomposting can be a cost-effective and sustainable technology for stabilization of industrial organic waste mixture of citronella bagasse and paper mill sludge. Significant increase in the dehydrogenase and urease activity accompanied by GI values of more than 80% in the end products indicated that the mature vermicompost is free of phytotoxic compounds. Results of the phytotoxicity test involving the seeds of *Abelmoschus esculentus* and *Solanum melongena* confirm suitable and efficient nature of vermicompost for using it as a potting media in the horticultural experiment as well as in organic farming practices.

Table 2 Correlation coefficients among dehydrogenase and urease activity and germination indices

	Dehydrogenase activity	Urease activity	GI of *A. esculentus*	GI of *S. melongena*
Dehydrogenase activity	1	0.99**	0.99**	0.99**
Urease activity	0.98*	1	0.99**	0.98**
GI of *A. esculentus*	0.99**	0.99**	1	0.99**
GI of *S. melongena*	0.99**	0.98**	0.99**	1

The correlation coefficients in the upper triangle belong to citronella bagasse (CB) and those in the lower triangle belong to citronella bagasse and paper mill sludge mixture (CB + PMS)

*Correlation is significant at the $P < 0.05$

**Correlation is significant at the $P < 0.01$

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Code availability Not applicable.

Data availability All data generated or analysed during this study are included in this article itself.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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