Effect of biochar supplementation on growth, reproduction, Enzyme level and Humification index on the vermicomposting with pressmud as substrate

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Abstract

Vermicomposting is the traditional method of biodegradation of organic compound into nutrient humus that helps in plant growth Pressmud is one of the waste products of the sugarcane industry with a rich organic composition. In this study, the pressmud is subjected to vermitransformation integrated with biochar. The biochar is amended at varied concentrations (0,2, 4 and 6%) with Pressmud and cow dung in three different compositions (1:1, 2:1 and 3:1) using *Eudrlius eugeniae* to generate an enhanced vermicompost. In the vermicompost combinations supplemented with biochar, there was increased growth and biomass of earthworms with the maximum observed in C7 (PM+CD (2:1) amended with 4% Biochar and C4 (PM+CD (1:1) amended with 6% biochar respectively. Microbial and Enzyme level analysis showed that a combination supplemented with biochar showed better results than the combinations without biochar. Overall Combination C3 (PM+CD (2:1) amended with 4% Biochar showed the best result in Microbial and enzyme analysis with the maximum achieved in 45 th day. Humification was also better in the biochar-supplemented combinations with the lowest humification index observed in C3 (0.6820 ± 0.027) and C4 (0.6912±0.031) pressmud+cow dung amended with 4% and 6% respectively in the final sample. This study shows that amendment of biochar at concentrations of 4% and 6% with pressmud as substrate showed better activity in the growth and reproduction of earthworms. The humification activity of the substrate was also maximum in the combination of C3 and C4 supplemented with 4% and 6% biochar respectively.

Key Words: vermicomposting, pressmud, vermitransformation, biochar, earthworm

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1. Introduction

India occupies an important position in the world's sugar economy and has surpassed Brazil as the leading sugar importer [1]. The juice is separated during sugar production from sugarcane and leads to the formation of waste. This waste is referred to as pressmud or filter cake. It has been widely known for its value as a plant organic fertilizer, containing a lot of plant nutrients. It not only improves soil quality but also provides organic nutrients that enhance crop growth. This byproduct poses disposal and impurity challenges and requires control measures and hygiene practices. According to Singh et al [2], the main rudiments of pressmud are humidity, fibre, raw wax, sugar, crude protein and nitrogen. The immeasurable by-product sourced from sugarcane refinement is traditionally discarded through either burning or burying.

Vermicomposting is a fantastic technology to convert the waste product of the sugar industry into valuable manure. The waste is consumed, digested, and transformed by earthworms under stringent microbial and earthworm activities into a finer moist more amenable microbial soil. Vermicomposting is a technique that transforms pressmud into a lucrative biofertilizer [3]. The earthworms break down the organic waste and get enriched with nutrients and growth-promoting agents through the process. This makes it an eco-friendly, socially responsible and economically viable approach. Earthworms help to convert the waste to compost which can improve plant growth and establish a sustainable agricultural system. [4]. Selecting the appropriate earthworm species is vital for successful vermicomposting. *Eudrilus eugeniae* is an effective choice [5] due to its characteristics like rapid growth, high reproduction rate and forbearance to diverse temperatures. Hence this study employed *Eudrilus eugeniae* for vermicomposting.

The recent utilization of biochar as a soil amendment has attracted considerable interest. A multitude of studies highlights the critical role of biochar in improving soil fertility and mitigating climate change. Biochar is noted for its efficient performance, stability, and generally alkaline characteristics, consisting of a carbon-rich solid produced through the slow pyrolysis of organic materials at high temperatures [6]. Vermicompost was treated with biochar to check its efficiency in the present study.

Earthworm reproduction, biomass production and survival are the main components of the vermicomposting process as reported by Bhat et al [7]. The different environmental factors which cause variation in the performance, numbers and distribution of earthworm in the environment. Parameters in the present study include the number and biomass of earthworms, number of cocoons and the amount of vermicompost produced.

The worm's digestive system contains bacterial microorganisms, nitrogen-fixing bacteria, enzymes and so on [8]. Earthworms help prepare natural materials for microbial decomposition and surface area for microbial activity. Bacteria, actinomycetes and fungi are the main microorganisms in vermicomposting [6]. Earthworms and

microbes produce enzymes which mineralize the organic matter through the vermicomposting process. Microbes play an important role in the fertilizer industry [9]. Therefore, it is not surprising that, just like earthworms, the enzymatic and microbial activity during vermicomposting is a good indicator of ecosystem health and soil fertility [10]. The present research focuses on the enzymes generated by earthworms and microorganisms throughout the vermicomposting process.

2. Materials and Procedures

2.1 Collection of raw material

For this study, the following materials were obtained. Pressmud gathered from Kallakurich Co-operative sugar mill in Moongithuraipattu, Thiruvannamalai, Tamil Nadu. Earthworm, *Eudrlius eugeniae* collected from VST natural farm in Kundumaranapalli, Hosur, Tamilnadu. Fresh cow dung sourced from a nearby animal farmhouse. Biochar procured from Greenfield Eco Solution.

2.2 Precomposting of pressmud

The press mud was laid out on a floor open to sunlight for five days. Then it was transferred to compost bags for precomposting which allows materials to decompose aerobically. This mixture was precomposed for 30 days to make it consumable for earthworms. Water was sprinkled periodically to maintain moisture content.

2.3 Vermicomposting of substrate

To study (Table 1) the impact of different combinations of the substrate (C1 to C12) with pressmud (PM), Cow Dung (CD) and Biochar (BC), 12 variations were examined. Each grow bag had a 5Kg capacity and contained various compositions of sugarcane trash, Press mud, cow manure and biochar. A random selection of 20 clitellated earthworms, *Eudrilus eugeniae* were placed into each of the 36 grow bags [11]. These earthworms were used to break down the organic matter and create vermicompost in a controlled environment, composting bags were kept at a constant temperature of 25 ± 3^0 C and left undisturbed to optimize vermicomposting. Moisture levels were maintained between 60-70% by regularly adding water. At the end of the study, earthworm counts, weight and cocoon numbers were recorded. The vermicompost was dried, sieved and stored for further analysis and characterization [7].

Table 1. Combination of substrate

S. No	Com bi-nations	Composition (2Kg/ver-mibed substrate)		Supplementary substrate compo-sition (Biochar)
		Ratio of Press mud	Ratio of cow dung	
1	C1	1	1	-
2	C2	1	1	2
3	C3	1	1	4
4	C4	1	1	6
5	C5	2	1	-
6	C6	2	1	2
7	C7	2	1	4
8	C8	2	1	6
9	C9	3	1	-
10	C10	3	1	2
11	C11	3	1	4
12	C12	3	1	6

2.4 Overview of vermicompost

The production and growth of biomass were measured in this study using the number and bio mass of earthworms, and the number of cocoons as an indicator of reproductive success. To determine each earthworm's unique biomass gain and cocoon susceptibility, the worms were carefully separated from the various worms cleaned with tap water and weighed using a digital electronic scale. Fig (1) represents the total number of earthworms in each of the 12-treatment group after the study.

2.5 Microbial analysis

Using the standard plate count method, the total colony forming units (CFU) of bacteria, fungi and actinomycetes in the vermibed materials was enumerated for the first and last sample [12,13]. The particulars can be found in Table 2. The gut contents of *Eudrilus eugeniae* were added to individual flask, which contained 99ml of sterile distilled water blanks. Subsequent serial dilutions were performed using 9 ml sterile distilled water as blanks [14]. Following the serial dilution process, 0.1 ml aliquots from the appropriate dilutions were spread onto serile nutrient agar plates. These plates were then incubated for a duration of 48 to 72 hours at a temperature of 37°C. Petri dishes exhibiting between 30 to 300 bacterial colonies were selected for the enumeration of the bacterial population. The bacterial population was quantified as colony-forming units (CFU) per gram of the sample [15].

A gram of each sample was placed in a sterile conical flask with nine milliliters of distilled water and subjected to shaking in a vortex mixer for a duration of 30 minutes [15]. The resulting stock was used to prepare multiple dilutions ranging from 10^{-1} to 10^{-7} using sterile distilled water. One ml of each diluted sample was then spread onto petri plates containing specific media for bacteria, fungi and actinomycetes. To ensure accuracy, each observation was repeated in three times.

Table 2. Media used to count the number of bacteria, fungi and actinomycetes in various vermibed substrates

Micro-organism	Media used	Dilution of the sample	Incubation in day(s)
Bacteria	Nutrient Agar Media	10^{-6}	1
Fungi	Martin's Rose Bengal Agar Media 103 3	10^{-3}	3
Actino-mycetes	Kenknight's Media	10^{-4}	7

2.6 Enzymatic analysis

The vermicompost was collected at the time intervals of 15 days (0^{th}, 15th, 30th, 45th, 60th, and 75th) and was subjected to enzymatic analysis. The activities of dehydrogenase and urease were assessed following the methodologies established by Cassida et al [16] and Tabatabai and Bremner [17]. Dehydrogenase activity was determined by measuring the formation of 2,3,5-triphenyl formazan (TPF) resulting from the reaction with 2,3,5-triphenyl tetrazolium chloride, and the results were expressed as μgTPF g^{-1} h^{-1}. Meanwhile, urease activity was evaluated by quantifying the ammonia released during the hydrolysis of urease, with results reported as mg NH^{4+} g^{-1} dw h^{-1}[18].

2.7 Statistical analysis

The results of the research on vermicompost characteristics and earthworm growth factors were presented as the average of three replicates, shown as mean ± S.D. Enzymatic and microbial parameters were analyzed on days 0,15,30,45,60 and 75 using SPSS software for Analysis of Variance at a 5% significance level, comparing treatment parameters initial and final values.

3. Result and discussion

3.1 Number, Biomass, reproduction of earthworm

The total amount of earthworms in all 12 treatment groups after the completion of the study is shown in (Fig.1a). In all groups, 10 earthworms were added. The number of worms in a vermicomposting mixture is influenced by the mixtures' fertility, the growth of the worms in the food environment and the worm's ability to adapt to the nutrients. The number of worms found in a 1:1 combination of press mud in the descending order is C3>C4>C1>C2. In the 2:1 combination, the specified order is C7>C8>C6>C5. In the 3:1 combination, the order is C11>C12>C10>C9. The highest quantity of earthworm population in press mud was C7 (225) and the lowest quantity was C11 (104). The significant increase in biomass can be attributed to the synergistic effects of organic waste, cow manure and nutrient additives, which enhance the palability and digestibility of the feed, leading to weight gain in the inoculated worms [19,20,21]. Conversely, the limited growth may be a result of the feed's low appeal, slow digestion rate and high carbon-nitrogen ratio [19].

The high biomass growth can be explained by the combined effects of organic waste, cow manure and nutrient supplements, which made the feed more palatable and easier to digest resulting in weight gain for the inoculated worms [19,20]. The weight of biomass is illustrated in (Fig 1b). The biomass of worms in 1:1 followed the order C3>C4>C2>C1. However, when the ratio is 2:1, the order becomes C7>C8>C6>C5. Finally, in a 3:1 ratio, the biomass pattern is C11>C12>C10>C9. The maximum biomass of earthworm in pressmud exhibit in C1 (34) and minimum was in C9(22.5). Karmegam et al [22], indicated that the difference in earthworm populations across various vermibeds can be linked to the variations in the nutrient composition of the vermicompost mixtures and C: N ratio. Numerous studies have demonstrated that the palatability and nutritional quantity of the substrates are essential to the growth and reproduction of earthworms, leading to discrepancies in earthworm biomass among different vermibeds [15,23,24,21]

The weight of vermicompost varies depending on the combination of materials used (Fig1 c). In a 1:1 combination, the decreasing weight trend was C1>C4>C2>C3.In a 2:1 combination, the decreasing trends were C6>C5>C8>C7. In a 3:1 combination, the trend was C10>C11>C12>C9. The most significant decline in vermicompost weight was observed in C7 (53%) while the least decline was observed in C1 (68%). As the earthworm population expands, it leads to a decrease in the mass of vermicomposting substrate through bio-oxidation and organic matter stabilization, which occurs due to the interaction between earthworm and microorganisms. Microorganisms are chiefly accountable for the biochemical decomposition of organic material, whereas earthworms significantly contribute to the fragmentation and conditioning of the substrate. This process enhances the surface area available for microbial proliferation and modifies its biological activity [25,26].

In descending order, the number of cocoons in a 1:1 combination of press mud is C3=C1>C2>C4. The order of C6>C7>C8>C5 is shown in a 2:1 combination. The order is C9>C10>C11>C12 in a 3:1 combination. (Fig 1d). The highest cocoon generation in press mud is observed at C9 (62) and the lowest amount at C4 (39). Cocoon production rate, cocoon initiation and cocoon weight in *E.eugeniae* were influenced by the biochemical properties of the substrate. Differences in cocoon production rates may be due to the influence of the quality of feed and culture [27,21]. Additionally, Biruntha et al 2020 [23] observed that decreased cocoon generation in *E.eugeniae* was caused by a high C:N ratio in the initial substrate. Based on the current study, adding biochar to a particular ratio of sugarcane waste and cattle dung significantly affect the growth and reproduction of earthworm-*Eudrilus eugeniae* and therefore, biochar addition to vermicomposting substrate should be provided is elucidated to obtain vermicompost with sufficient nutrients.

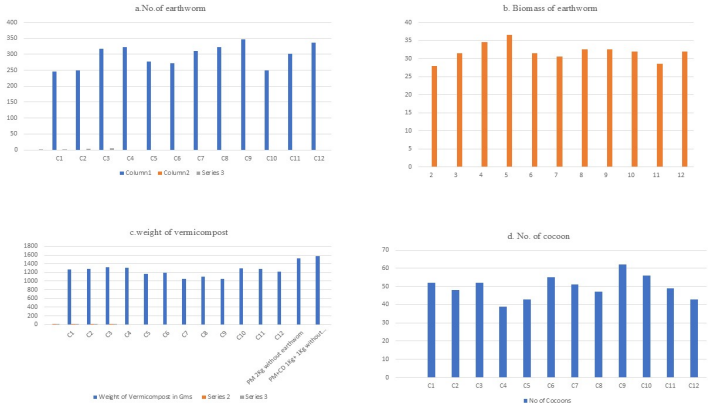

Fig.1. (a). Number of earthworm (b) Biomass of earthworm (c) Weight of vermicompost (d) No. of cocoons in various combinations of vermicompost.

3.2 Microbial analysis

The gut microbiome of earthworms consists of microbes which can even decompose and fix nitrogen that is excreted by nutrients in their vermicast [28]. The biochemical breakdown of the natural resource is primarily driven by microorganisms, including bacteria, fungi and actinomycetes [29] and regulate the ecological balance. Besides this, earthworms improve microbial activities which result in the increase of microbe population and its biodiversity. Therefore, the purpose of the current research was to identify the presence of bacteria, fungi and actinomycetes.

The fungal concentration (Fig 2a) developed from the beginning day to day 45, which was due to the abundance of organic material in the earthworm substrate. Substrate decomposition resulted in a shift in the microbial population between days 60 to day 75. Fungal populations, tending to be the most common composting agents, can withstand high temperatures and decompose a wide range of organic waste. Research has shown that earthworms particularly endogeic and epigeic species of earthworms consume fungi and stunt mycelium growth, causing fungi population and diversity to decline [30].

The isolation of bacterial strains from vermicompost distinguishes the microbes that are responsible for boosting soil fertility and allowing crops to grow. This will make us aware of the fact that bacteria participate in plant development and will help us to select the ones that are beneficial for vermicompost. The bacterial concentration of the present study (Fig.2b) showed a progressive increase from the initial day to 45 days. Later, it started declining until the end of the process like fungi. Monroy [31] conducted a trial which established a more than 75% reduction of total coliforms after vermicomposting over a period of two weeks employing E. foetida. The study conducted by Iria Vilar and associates [32] has shown that various kind of bacteria and fungi have gradually decreased in the entire process of developing compost and vermicompost from sewage sludge. Haung et al [33] studied to examine the bacterial and fungal populations trends during the vermicomposting process of green waste using *E. Foetida* and the result was that the decline in the number of fungi and bacteria after 60days in vermicompost production. The result of all these previous studies could be paralleled with the line of the present study.

Actinomycetes, species non-susceptible bacteria are impartial in destruction of organic matter in soil and sediments. The presently known species are able to create substances blocking the development of the detrimental microbes [34,35]. Among microbes, actinomycetes are the one that is known as the most promising as they enzymatically produce many different kinds like protease, amylases, cellulase and some antimicrobial substances. The result of the current study (Fig.2c) indicated that actinomycete increased gradually for 45 days and later eventually declined. It proliferates as they move through the worm's digestive tract that could be the cause of increased actinomycetes population [29]. When more resistant compounds were deleted at the process, a decrease in actinomycetes growth was predicted. Incooperating biochar in vermicomposting improves the physical, chemical properties of vermicompost and provides a suitable habitat for microorganisms and earthworms, which has a significant effect on the decomposition process by releasing microorganisms [36,37].

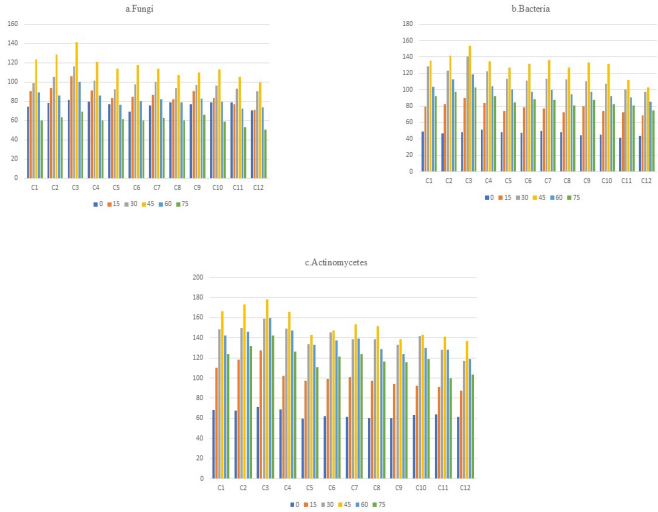

Fig 2 (a). Presence of fungi (b) bacteria (c) Actinomycetes in the vermicompost of different combinations

3.3 Enzymatic analysis

Microorganism possess the ability to synthesize a variety of enzymes like cellulase, dehydrogenase, phosphatase, urease etc. [38]. The enzymatic activities serve as indicators of both degradation process, reflection of microbial activity, and provide insights into maturity of the vermicompost [22,39,40,41]. This study focused on the presence of dehydrogenase, urease, acid phosphatase and alkaline phosphatase.

An enzyme such as urease, which is synthesized by the microorganisms to decrease the nutrient and energy holding capacity of the organic substances and nitrogen compounds. This enzyme converts urea to CO_2 and ammonium with its enzymatic reaction [42]. The urease enzyme turns out to be the vital enzyme that is engaged in transforming the organic nitrogen into inorganic nitrogen during vermicomposting by the means of which the plant absorb nitrogen readily. Throughout 75 days (Fig.3.a) of the study reported an increase in urease activity from the initial day due to low NH^{4+}content in the waste and later dropped from 60 to 75 days as a result of the increase amount of NH^{4+}content which hinders the urease activity. The use of carbon-based amendments such as biochar and straw increases urease activity by promoting the proliferation of urease producing microorganisms [41]. Ramalakshmi and colleagues [43] found out that vermicomposting of vegetable market waste reduced urease activity as time increased [44].

Dehydrogenase enzyme activity can be correlated with the metabolic state of fungal and bacterial population of soil or with maturity of vermicompost products [45]. In the initial stages of vermicomposting, dehydrogenase levels were high but decreased significantly as the decomposition progressed in this study. (Fig 3b). The increase in the microbial concentration of the pressmud, green manure and cow dung during the 30to 40 days of vermicomposting significantly enhanced the nutritious quantity of the vermicompost and enzymatic activity of dehydrogenase [20]. The corresponding study conducted by Karmegam and his colleagues [22] also showed that there was an increase in dehydrogenase activity, which was line with rise in the microbial population on vermicomposting with cow dung, green manure plant. paper mill sludge etc. and reduced later as the process continues. Decrease of feedstock substrate leads to decreased production of dehydrogenase [41].

Phosphorous together with nitrogen and potassium is a key macronutrient required for plant growth and yield. Phosphatase are hydrolases that break the ester relationship between the phosphate group and organic base of phosphate esters. Based on the optimum pH for the load, phosphatase is of two types acid and basic. The optimal effect of acid phosphatase is at an acidic pH around 6 and alkaline phosphatase show an optimal effect at an alkaline pH of around 11 [46,18]. The present study examined the presence of alkaline phosphatase and acid phosphatase. Alkaline and acid phosphatase enzyme showed high level of presence at the beginning of the process however, their activities started to decrease towards the end of the process. (Fig 3.c &d). The phosphatase in vermicompost may be the result of metabolized P-cycle substrates used by the earthworm and microorganism [44]. In a similar manner, phosphatase enzyme activities increased at the initial stage and subsequently decreased during the process of olive waste vermicomposting [47]. In accordance with Gong et al [48] who reported that biochar facilitates increased cellulose, alkaline phosphatase, dehydrogenase and urease activity in urban green waste.

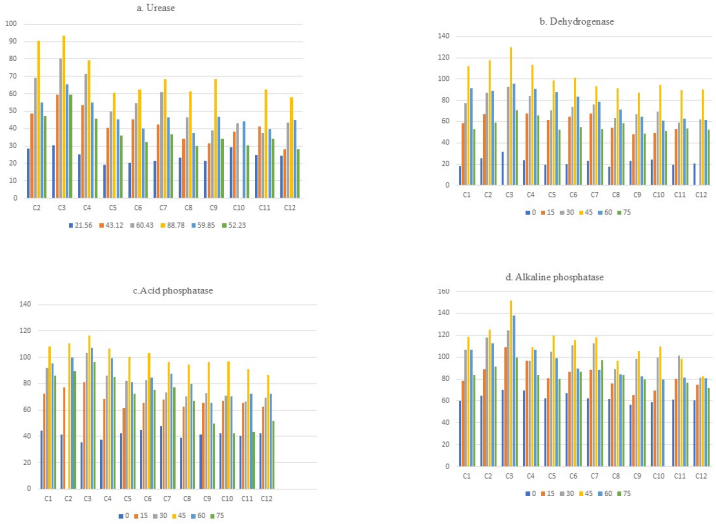

Fig.3. (a) Urease enzyme (b) Dehydrogenase enzyme (c) Acid phosphatase (d) Alkaline phosphatase present in the vermicompost.

3.4 Humification index

During humification, organic matter decomposes into stable humic substances that process is enhanced by addition of biochar [49,50]. Vermicompost maturity was evaluated through spectroscopic analysis of humification. Microbial enzyme releases humic acid production during this process [51]. The HI of the vermicompost of all combinations was analyzed during 0, 15, 30, 45,60 and 75th days of the study and HI decreases in the final values of the vermicompost when compared to Initial samples (Fig4). The lowest HI was observed in 75th day vermicompost in Combination C3 (1PM; ICD+4%BC) and C4 (1PM; ICD+6%BC) with 0.6820 and 0.6912 respectively. The decrease in the humification index throughout the composting process indicates an increase in aliphatic carbon in relation to aromatic carbon [51]. The amendment of biochar to the organic material facilitates its degradation and formation of enriched humus [50].

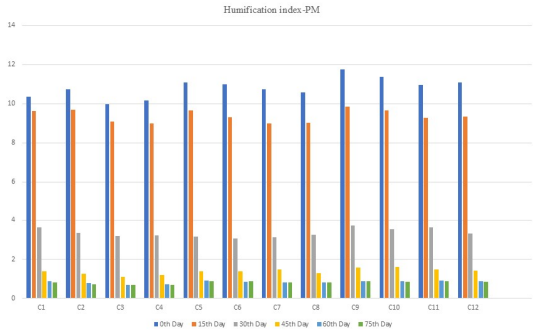

Fig.4. Humification index value of biochar amended pressmud and cowdung. Presented values are mean±S.D.

4. Conclusion

The present study shows that pressmud is an efficient substrate for the vermicomposting process. The addition of Biochar as a supplement with pressmud increased the growth and reproduction of earthworms in the vermicomposting process. Most of the combinations supplemented with biochar were shown to contain more earthworms and higher biomass than the combinations without biochar with the maximum observed in C7 and C4 respectively. The microbial analysis shows that the presence of analysed microbes was more in biochar-supplemented combinations and the same was observed in levels of different enzymes selected for this study. The supplementation of biochar increased the humification process of pressmud which was inferred from the lower humification index. This study shows that the amendment of biochar enhances the production of vermicompost and also helps in the proliferation of earthworms.

5. Declaration: The authors declare no competing interest.

References

1. Nihar, A., Patel, N. R., & Danodia, A. Machine-Learning-Based Regional Yield Forecasting for Sugarcane Crop in Uttar Pradesh, India. Journal of the Indian Society of Remote Sensing, (2022). https://doi .10.1007/s12524-022-01549-0.

2. Singh, A.K., Beer,K.,& Pal, A.K. Effect of vermicompost and biofertilizers on strawberry i: growth, flowering and yield. Annals of Plant and Soil Research 17 (2): 196-199 (2015)

3. Bhat, S.A., Singh, J., & Vig, A. P. Instrumental characterization of organic wastes for evaluation of vermicompost maturity. J Anal Sci Technol 8 (2017). https://doi.org/10.1186/s40543-017-0112-2

4. Moorthi, M., Nagarajan, K., & Senthil Kumar, A. Vermi-technology of organic solid waste with using earthworm Eudrilus eugeniae. The journal of Zoology Studies, 3(4), 48-51(2016).

5. Sujatha, K., Kowsalya, R., & Vimala Bangarusamy, R. V. Production of Vermicompost by Agrowaste using Eudrilus Eugeniae and Eisenia Fetida Species (Linn). Annals of the Romanian Society for Cell Biology, 819-827 (2021).

6. Liu, J., Jiang, B., Shen, J., Zhu, X., Yi, W., & Li, Y. Contrasting effects of straw and straw-derived biochar applications on soil carbon accumulation and nitrogen use efficiency in double-rice cropping systems. Agricult Ecosyst Environ 311, 107286 (2021). https://doi: 10.1016/j.agee.2020.107286.

7. Bhat, S.A., Singh, J., & Vig, A.P. Potential utilization of bagasse as feed material for earthworm Eisenia fetida and production of vermicompost. Springer plus, (2015). https://doi: 10.1186/s40064-014-0780-y.

8.Wang, N.,Wang, W., Jiang, Y., Dai, W., Li, P., Yao, D., Wang, J., Shi, Y., Cui, Z., & Cao, H.
Variations in bacterial taxonomic profiles and potential functions in response to the gut transit of earthworms (Eisenia fetida) feeding on cow manure. Sci Total Environ ,787,147392(2021). https://doi: 10.1016/j.scitotenv.2021.147392

9. Lakshmanan, R., & Muthunarayanan, V. Enzymatic analysis of natural and artificial banana leaf waste in vermicomposting and composting technique. Journal of Advanced Applied Scientific Research, 6, 2454-3225 (2016). https://doi:10.26418/protobiont.v8i1.30843

10. Gunjal, A.B., Waghmode, M.S., Patil, N.N., & Nawani, N.N. Significance of soil enzymes in agriculture, in Smart Bioremediation Technologies. Microbial Enzymes, 159–168 (2019).

11. Wrushali, M., Meshram, N.A., Dalvi, V.V., Shigwan, A.S., Tripathi, V.D., Rane, A.D., & VG. Physicochemical and biological characterization of vermicompost prepared from different residues of agroforestry component. Applied Biological Research, 21(3),255-264 (2019). https://doi. 10.5958/0974- 4517.2019.00034.X

12. Subba Rao, N. S. Soil microorganisms and plant growth (third edition), 77 – 293, oxford and IBH publishing company, New Delhi (1995).

13. Kannan, N. Physiological characteristics of microorganisms. Laboratory Manual in General Microbiology, Palani Paramount Publication, Palani.1-316 (1996)

14. Maruthamuthu, S., Kumar, B.,Shanmugavel, R., Balakrishnan, A., Palanichamy, S., Chandrasekaran, M., Subramanian, P., & Palaniswamy, N. Microbial Corrosion in Petroleum Product Transporting Pipelines. Industrial & Engineering Chemistry Research, 50, 8006–8015 (2011). https://doi:10.1021/ie1023707.
15.Ananthavalli, R., Ramadas, V., Paul, JA.J., Selvi, B.K., & Karmegam, N. Vermistabilization of seaweeds using an indigenous earthworm species, Perionyx excavatus (Perrier) Ecological engineering. Journal Elsevier, 130, 23-31 (2019). https://doi:10.1016/j.ecoleng.2019.02.001
16. Casida, L., et al.: Soil dehydrogenase activity. Soil Science, 98(6),371-376, (1964).
17. Tabatabai, M.A., & Bremner, J.M. Assay of urease activity in soils. Soil Biology and Biochemistry,Volume 4, Issue 4, 479-487(1972). https://doi.org/10.1016/0038-0717(72)90064-8
18. Jayakumar, M., Emana, A.N., Subbaiya, R., Ponraj, M., Ashok Kumar, K.K., Muthusamy, G., Kim, W., & Karmegam, N. Detoxification of coir pith through refined vermicomposting engaging Eudrilus eugeniae. Chemosphere,291(Pt 2), 132675 (2021). https://doi: 10.1016/j.chemosphere.2021.132675.
19. Suthar, S. Pilot-scale vermireactors for sewage sludge stabilization and metal. Ecol Eng, 36,703-712 (2009. https://doi.org/10.1016/j.ecoleng.2009.12.016
20.Balachandar, R., Baskaran, L., Yuvaraj, A., Thangaraj, R., Subbaiya, R., Ravindran, B., Chang, S.W., & Karmegam, N. Enriched pressmud vermicompost production with green manure plants using Eudrilus eugeniae. Bioresour Technol, 299, Article 122578 (2020). doi: 10.1016/j.biortech.2019.122578.
21. Yatoo, A. M., Bhat, S. A., Ali, M. N., Baba, Z. A., & Zaheen, Z. Production of Nutrient-Enriched Vermicompost from Aquatic Macrophytes Supplemented with Kitchen Waste: Assessment of Nutrient Changes, Phytotoxicity, and Earthworm Biodynamics. Agronomy, 12(6), 1303 (2022). https://doi.org/10.3390/agronomy12061303
22. Karmegam, N., Vijayan, P., Prakash, M., & Paul, J.A.J. Vermicomposting of paper industry sludge with cowdung and green manure plants using Eisenia fetida: a viable option for cleaner and enriched vermicompost production. J. Clean. Prod, 228,718-728 (2019). https:// doi: 10.1016/j.jclepro.2019.04.313.
23. Biruntha, M., Karmegam, N., Archana, J., Selvi, B.K., Paul J.A.J., Krishnan, B.M., Chang, S.W., & Ravindran,B. Vermiconversion of biowastes with low-to-high C/N ratio into value added vermicompost. Bioresour Technol, 297, 122398 (2020). doi: 10.1016/j.biortech.2019.122398
24. Balachandar, R., Biruntha, M., Yuvaraj, A., Thangaraj, R., Subbaiya, R., Govarthanan, M., Kumar, P., & Karmegam, N.Earthworm intervened nutrient recovery and greener production of vermicompost from ipomoea staphylina–an invasive weed with emerging environmental challenges.Chemosphere, 263,Article 128080 (2021).https://doi.org/10.1016/j.chemosphere.2020.128080
25.Domínguez, J. State of the art and new perspectives on vermicomposting research. In: Edwards, C.A. (ed.), Earthworm Ecology, 2nd Edition, 401–424 (2004). https://doi:10.1201/9781420039719.ch20
26.Amaravathi, G., & Reddy, M.R. Environmental factors affecting vermicomposting of Municipal solid waste. International journal of pharmacy and biological sciences, 5(3), 81-93 (2015). www.ijpbsonline.com

27. Coulibaly, S.S., Kouassi, K.I., Koffi, K.K., & Zoro, B.I.A. Effect of compost from different animal manures on maize (Zea mays) growth. Journal of Experimental Biology and Agricultural Sciences, 7(2), 178-185 (2019). https://doi:10.18006/2019.7(2).178.185
28. Pathma, J., & Sakthivel, N. Microbial diversity of vermicompost bacteria that exhibit useful agricultural traits and waste management potential. SpringerPlus,1,26, (2012). https://doi: 10.1186/2193-1801-1-26
29. Emperor, G.N., & Kumar, K. Microbial Population and Activity on Vermicompost of Eudrilus eugeniae and Eisenia fetida in Different Concentrations of Tea Waste with Cow Dung and Kitchen Waste Mixture. International journal of current microbiology and applied sciences, 4, Number 10, 497-506 (2015). http://www.ijcmas.com
30.Hoeffner, K., Monard, C., Santonja, M., & Cluzeau, D. Feeding behaviour of epianecic earthworm species and their impacts on soil microbial communities. Soil Biol Biochem,125, 1–9 (2018). https:// doi: 10.1016/j.soilbio.2018.06.017.
31. Monroy, F., Aira, M., & Domínguez, J. Reduction of total coliform numbers during vermicomposting is caused by short-term direct effects of earthworms on microorganisms and depends on the dose of application of pig slurry. Sci Total Environ,407,5411–6 (2009). https://doi: 10.1016/j.scitotenv.2009.06.048.
32.Villar, I., Alves, D., Pérez-Díaz, D. Changes in microbia dynamics during vermicomposting of fresh and composted sewage sludge. Waste Manag, 48, 409-417 (2015). https://doi: 10.1016/j.wasman.2015.10.011
33. Huang, K., Li, F., & Wei, Y. Changes of bacterial and fungal community compositions during vermicomposting of vegetable wastes by Eisenia foetida. Bioresource technol,150, 235-41(2013). https:// doi: 10.1016/j.biortech.2013.10.006.
34. Ryandini, D., Radjasa, O.K., & Oedjijono. Isolate Actinomycetes SA32 Origin of Segara Anakan Mangrove Rhizosphere and Its Capability in Inhibiting Multi-Drugs Resistant Bacteria Growth. J Microb Biochem Technol, 10, 1-7, (2018).https://doi:10.4172/1948-5948.1000386
35. Lestari, S., Mukarlina., & Kurniatuhadi, R. Identifikasi dan Deteksi Aktivitas Daya Hambat Bakteri Actinomycetes yang Diisolasi dari Tanah Gambut di Desa Tajok Kayong Kalimantan Barat. Protobiont, 8, 13-9 (2019). https:// doi:10.26418/protobiont.v8i1.30843
36. Jindo, K., Sánchez-Monedero, M.A., Hernández, T., García, C., Furukawa, T., Matsumoto, K., Sonoki, T., & Bastida, F. Biochar influences the microbial community structure during manure composting with agricultural wastes. The Science of the Total Environment, 416, 476–481 (2012). https://doi:10.1016/j.scitotenv.2011.12.009
37. Khan, M.B., Cui, X., Tang, L., Lu, M., Cao, X., Sahito, Z., Hamid, Y., Hussain, B., Yang, X., & He, Z. New insight into the impact of biochar during vermi-stabilization of divergent biowastes: Literature synthesis and research pursuits. Chemosphere. 238. 124679(2019). (2019). https:// doi: 10.1016/j.chemosphere.2019.124679.
38. Gusain, R., & Suthar, S. Vermicomposting of duckweed (Spirodela polyrhiza) by employing Eisenia fetida: changes in nutrient contents, microbial enzyme activities and earthworm biodynamics. Bioresour Technol, 311, Article 123585 (2020a). https://doi: 10.1016/j.biortech.2020.123585
39. Santana, N.A., Jacques, R.J.S., Antoniolli, Z.I., Cordeiro, H.M., & Domínguez, J.

Changes in the chemical and biological characteristics of grape marc vermicompost during a two-year production period. Appl Soil Ecol, 154, Article 103587 (2020). https://doi :10.1016/j.apsoil.2020.103587
40.Tiquia, S. Evolution of extracellular enzyme activities during manure composting. J Appl Microbiol,92(4),764-75 (2002). https:// doi: 10.1046/j.1365-2672.2002.01582.x.
41. Enebe, M.C., & Erasmus, M. Mediators of biomass transformation – A focus on the enzyme composition of the vermicomposting process. Environmental challenges, Volume 12,100732 (2023). https://doi:10.1016/j.envc.2023.100732
42. Maroney, M.J., & Ciurli, S. Non redox nickel enzymes. Chem. Rev, 114, 4206-4228 (2014). https:// doi: 10.1021/cr4004488
43. Lakshmi, Ch.S.R., Rao, C.P., Sreelatha, T., Madhavi, M., Padmaja, G., & Sireesha, A. Changes in enzyme activities during vermicomposting and normal composting of vegetable market waste. Agric Sci Digest, 34(2),107-110 (2014). https://doi:10.5958/0976-0547.2014.00025.1
44.Shanthi, K. Comparative assessment in enzyme activities during vermicomposting of bio-degradable wastes. International Journal of Academic Research and Development, Volume 3, Issue 2, 1227-1230, (2018).
45.Yadav, A., Suthar, S., & Garg, V.K. Dynamics of microbiological parameters, enzymatic activities and worm biomass production during vermicomposting of effluent treatment plant sludge of bakery industry. Environ Sci Pollut Res, 22,14702–14709 (2015). https://doi.org/ 10.1007/s11356-015-4672-7
46. Dotaniya ML, Aparna K,Dotaniya CK, Singh M,Regar KL(2019) Role of Soil Enzymes in Sustainable Crop Production, in Enzymes in Food Biotechnology. 569-589. doi:10.1016/B978-0-12-813280-7.00033-5
47. Benitez, E., Sainz, H., &Nogales, R. Hydrolytic enzymes of extracted humic substances during the vermicomposting of a lignocellulosic olive waste. Bioresour. Technol. 96, 785-790 (2005). https://doi: 10.1016/j.biortech.2004.08.010.
48. Gong, X., Zou, L., Wang, L., Zhang, B., & Jiang, J. Biochar improves compost humification, maturity and mitigates nitrogen loss during the vermicomposting of cattle manure-maize straw. J Environ Manag, 325, 116432 (2022). https://doi.org/10.1016/j.jenvman.2022.116432.
49.Gong, X., Cai. L., Li, S. Bamboo biochar amendment improves the growth and reproduction of Eisenia fetida and the quality of green waste vermicompost. Ecotoxicol Environ Saf,156,197–204 (2018). https://doi.org/10.1016/j.ecoenv.2018.03.023
50. Ashok Kumar, K., Subalakshmi, R., Jayanthi, M., Abirami, G., Vijayan, D.S., Venkatesa Prabhu, S., & Baskaran, L. Production and characterization of enriched vermicompost from banana leaf biomass waste activated by biochar integration. Environ Res, 15,219,115090 (2023). https://doi:10.1016/j.envres.2022.115090.
51. Ganguly, R.K., & Chakraborty. S. K. Assessment of qualitative enrichment of organic paper mill wastes through vermicomposting: humification factor and time of maturity. Heliyon, 5(5), e01638, (2019). https://doi.org/10.1016/j.heliyon.2019.e01638