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Effective Utilization of Fly Ash for Vermicompost Production by Employing *Eisenia fetida*

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Fly ash; *Eisenia fetida*; Enzyme activity; Antioxidant assay

ABSTRACT

Thermal Power plants cater the energy needs in many of the countries across the globe, but they indeed pose health hazard to the atmosphere by the release of pollutants such as fly ash, particulate matter, dust, smoke-laden with gaseous pollutants etc., As it is mandatory to meet the energy demand of the increasing population, it is also important to manage the waste produced as the result of these industries. Though there exist various methodologies to manage the waste, vermicomposting is one of the cost-effective and simple techniques available to manage many of the solid waste emanated from different industries. Hence, this study was made to manage the fly ash waste by vermicomposting technique after the addition of carbon substrate namely, the cow dung in different rations such as 1:1, 1:4, 4:1, 2:3 and 3:2. The organism selected for the research was *Eisenia fetida.* The number of days for the process was about 60 days. The physico-chemical changes were monitored throughout the study period at regular intervals. The bacterial strains were isolated from the end product, namely the vermicast or vermicompost. Their enzyme activity was also checked and the end product was characterized using FTIR and XRD. Of the different proportions, 1:4 was found to be a suitable proportion in terms of the parameters checked.

INTRODUCTION

Excessive growth of population leads to rapid urbanization and industrialization which increases the demand for electricity throughout the world. In most of the developing countries, power has been observed as an important tool of growth (Usmani et al. 2017). The power generating facilities results in the production of fly-ash, which contains 0.01 to 100 mm particles mimicking glass (Davison et al. 1974). In fly ash trace amount of oxides, hydroxides, carbonates, silicates, and sulphates of calcium, iron, aluminium, and other metals are present (Adriano et al. 1980). During the 20th century, the production of fly ash in India was about 95 million tons and it has reached up to 112 million tons during 2005-2006. In 2009-2010 fly ash production of the country reached 180 million tons (Chattopadhyay & Bhattacharya 2010). For the year of 2011-12, the electricity generated was about 985 terawatt hours (TWh) (SEPSI 2000). During the electricity generation, combustion of powdered coal leads to the production of fly ash, one of the most abundant waste materials. As per MOEF (2007) at the end of the 11th 5-year plan, about 150-170 million tons of fly ash was expected to be generated per year.

Fly ash is reported to be present in the atmosphere for a reasonable time and is reported to cause varied health effects (T.E.R.I. 1998). It poses a threat to the environment too. It blocks natural drainage and reduces the pH, potability of water and makes it turbid. Fly ash further affects the process of photosynthesis and damages the plants too (Gupta et al. 2005). In the coming years, the disposal of fly ash would be a challenging task. Though the accepted disposal methods include dumping the fly ash in settling ponds, stockpiling, and in landfills, such disposal methodologies have their limitations too. The disposed fly ash in landfills contaminates the soil, groundwater and surface water (Pandey et al. 2009).

In India, the fly ash generated is being used massively for the construction purposes, which includes the production of bricks and cement and it is also being used for the formation of embankments too. Further, it is utilised for the land reclamation by filling the low-lying areas and for the reclamation of wasteland too. It is also being utilised for mine filling too.

Solid waste management is a problem which needs immediate attention as far as Indian cities are concerned. (Karthika et al. 2014). It was reported that vermicomposting would be a feasible, low-cost and an eco-friendly technique for organic waste management (Logsdson 1994). It has been demonstrated by several workers regarding the ability of the earthworms to decompose the organic wastes including animal wastes, crop residues, sewage sludge and industrial refuse (Edwards 1998). Some of the earthworm species are identified as heavy metal tolerant species. They were found to detoxify the municipal solid waste including industrial sludge through the process of vermicomposting (Saxena et al. 1998). Vermicomposting is capable of handling fly ash from very low to high quantities. It is a process with significant advantages such as low-cost and comparatively simple. Hence it could be practised in both small scale and large scale too. The vermicast was found to contribute many nutrients to the soil facilitating the plant growth (Abbasi & Ramasamy 1999, Ismail 1997).

Based on the review made regarding the fly ash disposal, we attempted in this study to convert the fly ash as organic fertilizer by vermicomposting which benefits the environment. The present study was made to estimate various physicochemical parameters from the initial till final vermicompost (0^{th} day to 60^{th} day) samples. Further, the vermicompost was characterised using FTIR and XRD and the antioxidant enzymes of the earthworm were determined. The study also includes the isolation of the bacterial strains and to perform the enzyme assays for amylase, protease, lipase and cellulase.

MATERIALS AND METHODS

Waste collection: The fly ash waste was obtained from Neyveli, Tamil Nadu, India. The carbon substrate namely cow dung was obtained near Bharathidasan University, Tiruchirappalli from a farm. The proportion of waste subjected to vermicomposting is listed in the Table1.

Physicochemical characterization of the vermicompost: The vermicompost samples from different ratios such as 1:1, 1:4, 4:1, 3:2 and 2:3 were drawn on initial, 15^{th}, 30^{th}, 45^{th} and 60^{th} day. The physico-chemical characters such as pH, electrical conductivity (EC), total organic carbon (TOC), total organic matter (TOM), total phosphorus (TP) and total potassium (TK) of the samples were analysed (Tandon 2009).

Microbial study: Serial dilutions of 1 g of the sample up to 10^{-3} to 10^{-7} dilution were made and plated on nutrient agar to enumerate the bacterial colonies (Yasir et al. 2009). Further from the isolated bacterial colonies, genomic DNA was isolated using DNA extraction mini kit (Real Biotech Corporation, Chennai). The universal primers (27 F 5′-AGAGTTTGATCMTGGC TCAG-3′ and 1492R 5′-TAC GGYTACCTTGTTAC GACTT-3′) were used for PCR amplification (Vivas et al. 2009).

Enzyme assay: The activity of SOD refers to measure the tendency to inhibit the pyrogallol oxidation in an alkaline environment. The method utilized was of Giannopolitis & Ries (1977) with slight modification. The catalase function was estimated as per Song et al. (2009). The activity of GPX was assayed as per Gunzler & Flohe (1985). The activity of GST was assayed according to the method of Saint Denis et al. (1998). The quantification of protein was done as per Bradford (1976).

Fourier transform infrared (FTIR): About 10 mg of dried end sample along with 500 mg of (KBr) was pelletized for 5 min with a hydraulic press and the resultant spectrum was recorded employing PerkinElmer Spectrum Version 10.03.09 (Zainab et al. 2009).

RESULTS AND DISCUSSION

Physico-chemical characteristics of fly ash waste: The vermicompost was prepared from the mixture containing cow dung, different quantities of fly ash waste, along with the earthworm population. The vermicompost was analysed for the parameters such as pH, EC, alkalinity, TOC, TOM, phosphorous and potassium. The values are presented in Fig. 1.

In the current scenario, waste management is a prime necessity. Agricultural soils lack nutrients due to the increased usage on fertilisers, pesticides thereby losing the native organisms and fertility. On the other hand, various nutritive wastes are improperly disposed and they are wasted. It is reported by (Bhide et al. 1994) that about 700 million tons of organic wastes are either landfilled or burnt. Instead, such wastes could be effectively managed by vermicomposting in-

Table 1: Waste subjected to vermicomposting.

S.No	Samples	Ratio of the sample	Wt. of the sample (g)
1	Fly Ash + Cow Dung	1:1	250 + 250 (500g)
2	Fly Ash + Cow Dung	1:4	100 + 400 (500g)
3	Fly Ash + Cow Dung	4 :1	400 + 100 (500g)
4	Fly Ash + Cow Dung	2:3	200 + 300 (500g)
5	Fly Ash + Cow Dung	3:2	300 + 200 (500g)

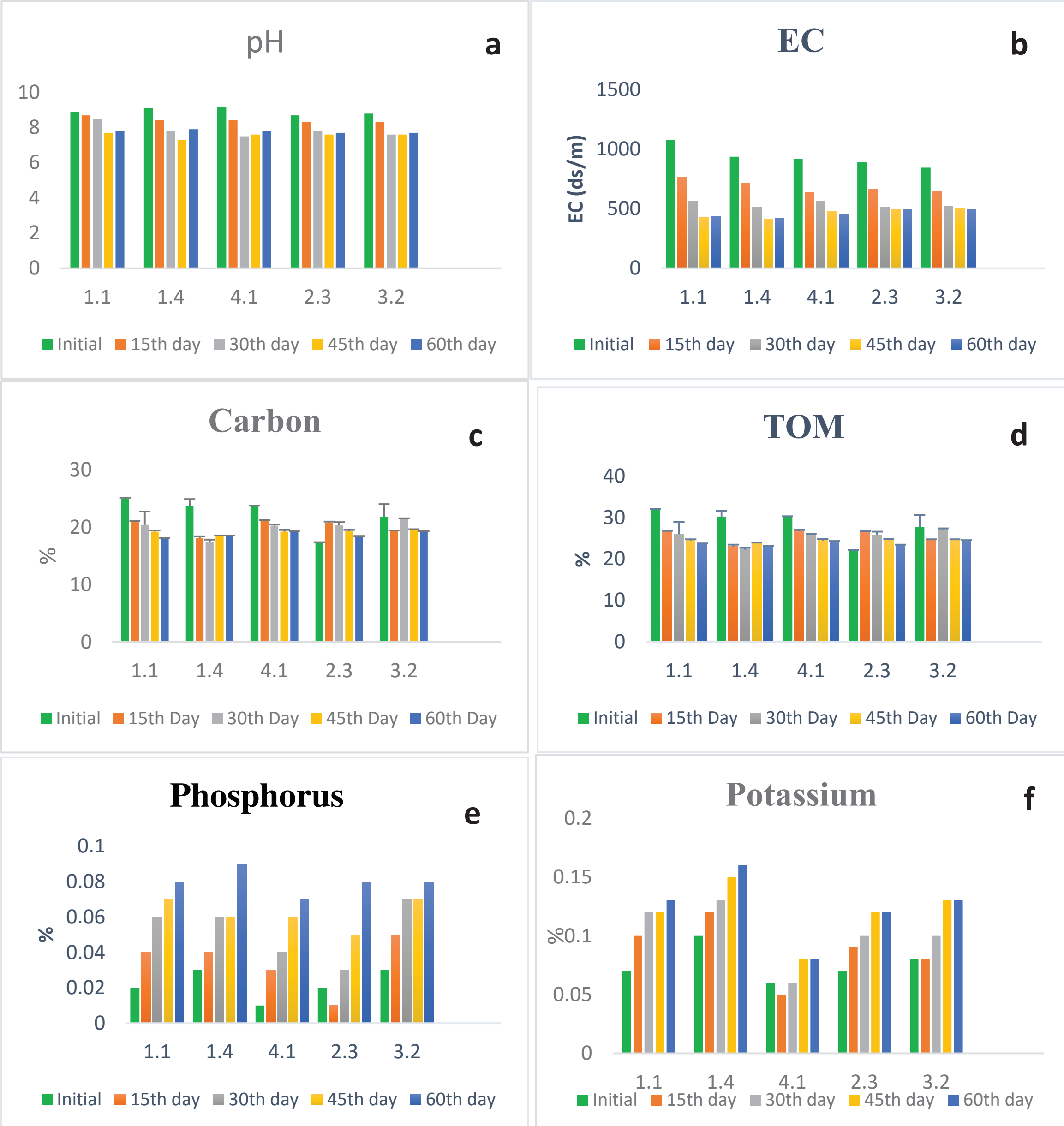

Fig. 1: (a–f) Physico-chemical properties fly ash waste vermicompost.

volving both earthworms and microbes efficiently. However, composting and vermicomposting differs significantly due to the pronounced activity of the earthworms (Gandhi et al. 1997). Hence this work includes the management of fly ash by vermicomposting. The entire process of vermicomposting of ash waste was carried out with the addition of cow dung and earthworm. The process was performed for 60 days. During the process, the variations in the physicochemical parameters were observed.

pH is an important parameter which is used to find the suitability of the vermicompost as manure. Hogg et al. (2002) have reported that the pH of about 6.0-8.5 would be suitable for promoting plant growth in most of the soils. Following the experiments, it was clear that the pH was reduced compared to the initial value, which may be due to the fulvic and humic acids produced during the vermicomposting process. Similar results were reported by Atiyeh et al. (2002). The EC values have decreased significantly in final vermicompost as follows in different ratios of wastes: in 1:1 ratio, the reduction was by 60%, in 1:4 by 55%, in 4:1 by 51%, in 2:3 ratio it was by 44% and in 3:2 ratio the reduction was about 40.6%. The decrease in EC could be attributed to the volatilisation of ammonia accompanied by the precipitation of certain salts in the dissolved state produced including ammonium salts in the final product (Singh et al. 2016). Lim et al. (2012) have noticed a remarkable decrease in the electrical conductivity of vermicompost produced from the sludge of the beverage industry.

During the decomposition of the waste by earthworms, the total organic carbon undergoes a reduction as per Pattnaik & Reddy (2010). Similar result was observed in this study during the decomposition of fly ash too. In this study, Fig. 1c shows that the TOC has highly reduced during the vermicomposting, the percentage of reductions in TOC was around 5-23.5 in all the treatments. The reduction was higher in the ratio 1:4, which was about 26%. Such loss of carbon in the form of carbon dioxide results in an increase in the nitrogen production (Crawford 1983) which could be attributed to the earthworm and microbial respiration. This study too showed a reduction of 26% in 1:4 ratio vermicomposting, which may be attributed to the presence of consortia of microbes Fig. 1d.

During the vermicomposting process, the total phosphorus content in the feed mixture got significantly increased. Data showed in Fig. 1e revealed that the percentage of total phosphorus got increased by 15-18% in all the treatments. The increase was higher in 1:4 (19 %) vermicompost for fly ash waste. The increase in the total phosphorus may be due to the presence and function of the earthworms and phosphate solubilizing microbes during the processing of the wastes which includes the mineralization of phosphorus (Ravindran et al. 2008). Similar report was reported by Satchell & Martin (1984). They stated that both earthworms and microbes could attribute the increase in total phosphorus. Similar trend has been reported earlier by Rajesh Banu et al. (2008). Along with an increase in the phosphorus content, a mild increase in the potassium was also noticed (08-13%) in all the treatments. The increase was higher in 1:4 (16%) vermicompost for fly ash waste (Fig. 1f). The increase in potassium is normally attributed to the acid-producing gut microbes of the earthworm as per Suthar (2006). However, Kaushik & Garg (2004) has interpreted that the increase in potassium may be due to the draining of water from the feed mixture. As a result of vermicomposting MSW, Kaviraj & Sharma (2003) reported an increase (10%) of total potassium by *Eisenia fetida* and by *Lampito mauritii* (5%) and it was attributed to the presence of microflora.

Isolation of genomic DNA and 16s rRNA: The isolated bacterial strains were subjected to genomic DNA isolation and PCR and the amplification results are shown in Figs. 2a and b.

Bacterial enzyme activity: The isolated bacterial strains were subjected to enzyme activity such as protease, amylase, cellulase and lipase and the results are as shown in Fig. 3.

Enzyme assay: The medium used for the enzyme assay includes starch agar medium, skim milk agar medium, LB tributyrene and Czapek mineral salt agar for amylase, protease, lipase and cellulose respectively. The plates were observed for the zone of clearance called halo. The appearance of clear halos around the bacterial strains confirmed

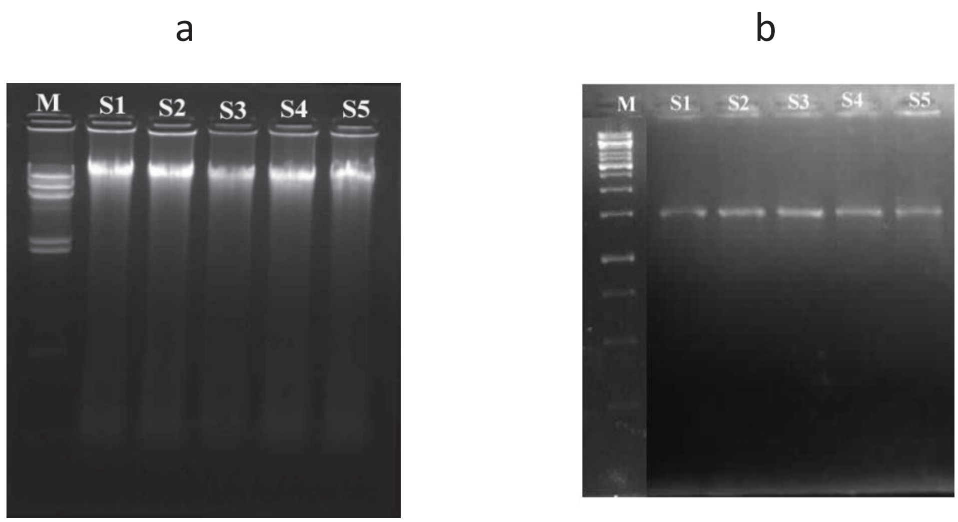

Fig. 2: a. Genomic DNA and b. PCR profile.

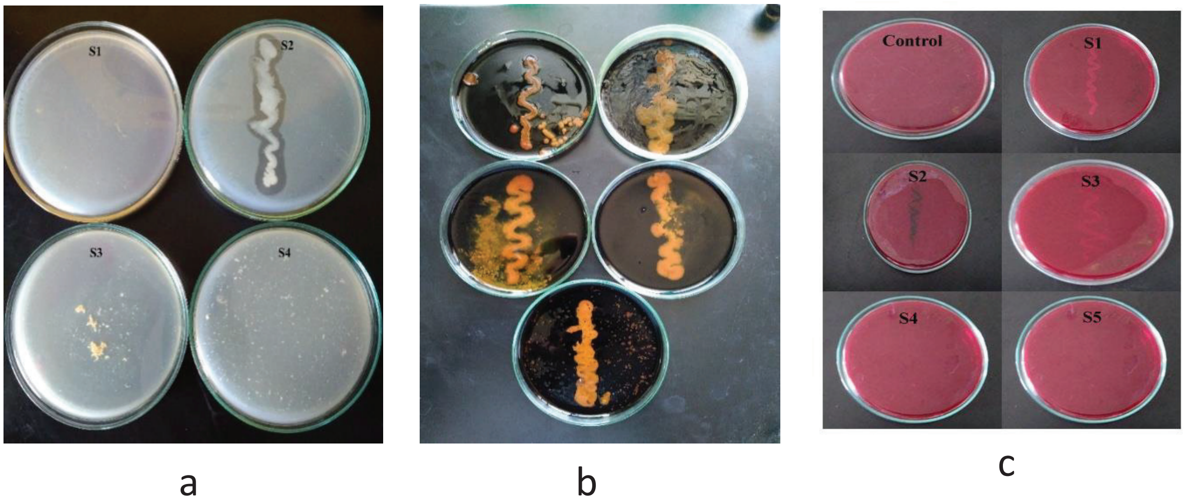

Fig. 3: Isolation of a- Protease, b- Amylase and c- Cellulase producing bacteria from fly ash vermicompost.

Table 2: Enzyme assay.

Enzymes	Strain 1	Strain 2	Strain 3	Strain 4	Strain 5
Amylase	+	+	+	+	+
Cellulase	+	+	+	-	-
Lipase	-	-	-	-	-
Protease	-	+	-	-	-

the respective enzyme production of the strains (Figs. 3 a, b, c and Table 2).

Antioxidative assay: The antioxidative assays such as Glutathione Peroxidase (GPx), Glutathione S-Transferase (GST), Super Oxide Dismutase (SOD) and Catalase (CAT) were performed for the earthworm samples. The results are represented in Fig. 4.

The biochemical responses of *E. fetida* during vermicomposting of the waste containing fly ash and butte sheet are shown in Fig. 4. There was an increase noticed in CAT and SOD activities and a reduction was noticed in the GST after 60 days. No significant change was noticed for GPx. However, oxidative stress was noticed in the earthworm which may be due to the continuous consumption of fly ash resulting in higher ROS production along with the subduing of the natural antioxidant enzymes (Nel et al. 2006). The authors have reported that during such process, subcellular injuries (protein denaturation, membrane damage, DNA damage, etc.) may occur. But the enzymes were reported to protect cells against the effects of ROS (Sharma et al. 2012). However, increased SOD and CAT activity may be related to the toxicity of fly ash.

Reports exist to prove that SOD catalyses the superoxide anion dismutation and the cells are protected by the elimination of H_2O_2 due to the presence of Catalase (Saint-Denis et al. 1998). Similarly, the reduction in CAT and SOD activity might be attributed to the SOD inactivation by singlet oxygen, hydrogen peroxide and peroxyl radicals (Fatima et al. 2007, Sandrini et al. 2008). Similarly, reduction in SOD and CAT was reported by Fatima et al. (2007) in the ASDI-exposed group representing a common cascade of events and Ribera et al. (2001) reported CAT reduction in the earthworm (*E. fetida*) exposed to carbaryl, an insecticide.

The hypothesis framed is that the fly ash degradation has posed stress to the earthworms thereby damaging the cells and its DNA. Similarly, the variation noticed in the enzyme activities were in accordance with the reports of Honda et al. (2000).

FTIR analysis for vermicompost: The vermicompost prepared were subjected to FTIR analysis and the results are shown in Fig. 5.

FTIR analysis provides the details about the functional groups present which thereby informs us of the degree of waste stabilization (Ravindran et al. 2008). FTIR spectra of the

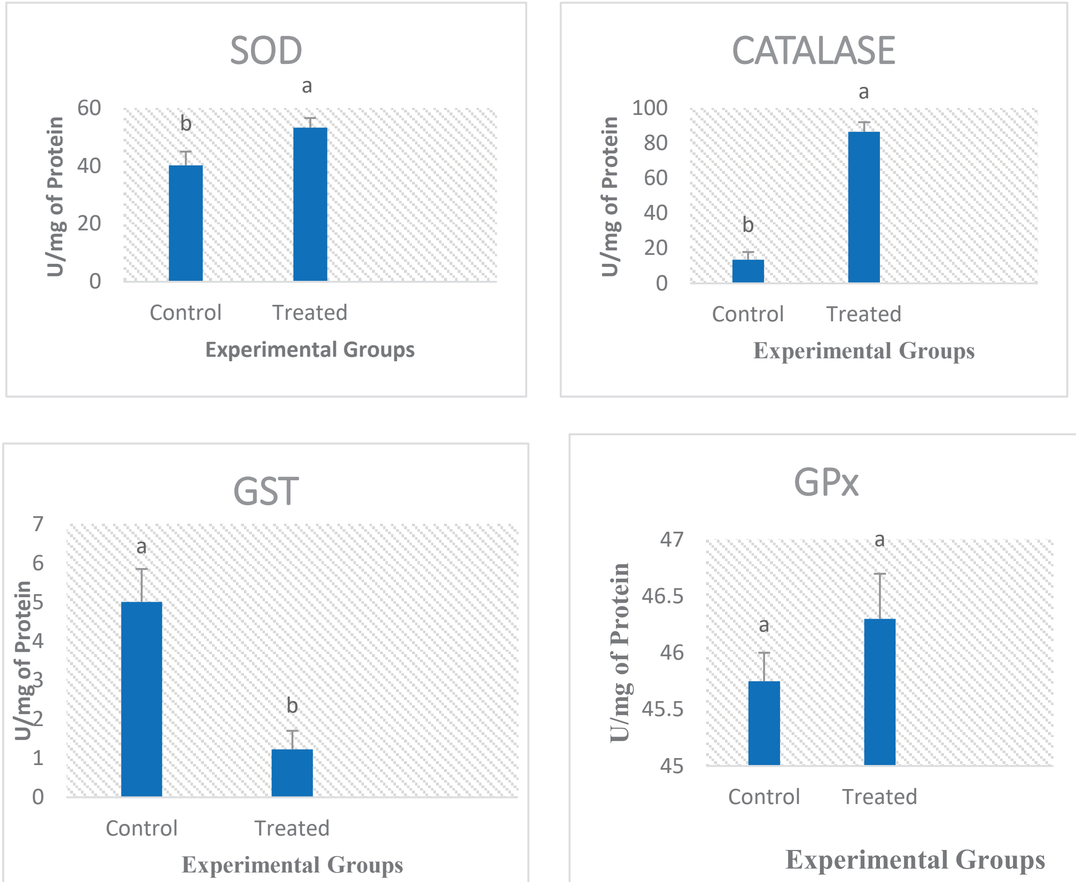

Fig. 4: Biochemical responses SOD, CAT, GST and GPx activity of *Eisenia fetida* utilized for vermicomposting of Fly ash waste. Values were expressed as mean ± SD (N=6). Bar with different alphabets are significantly different from each other and with same alphabets have insignificant changes (P<0.05).

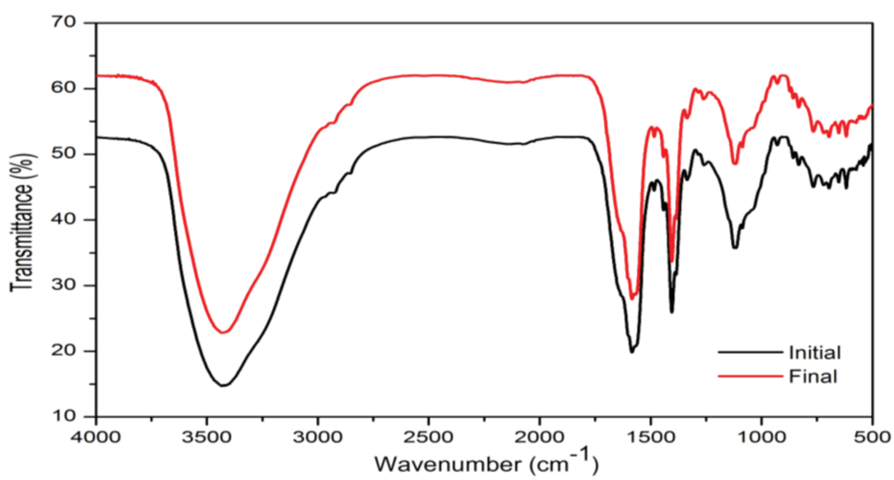

Fig. 5: FTIR pattern of the initial and final vermicompost.

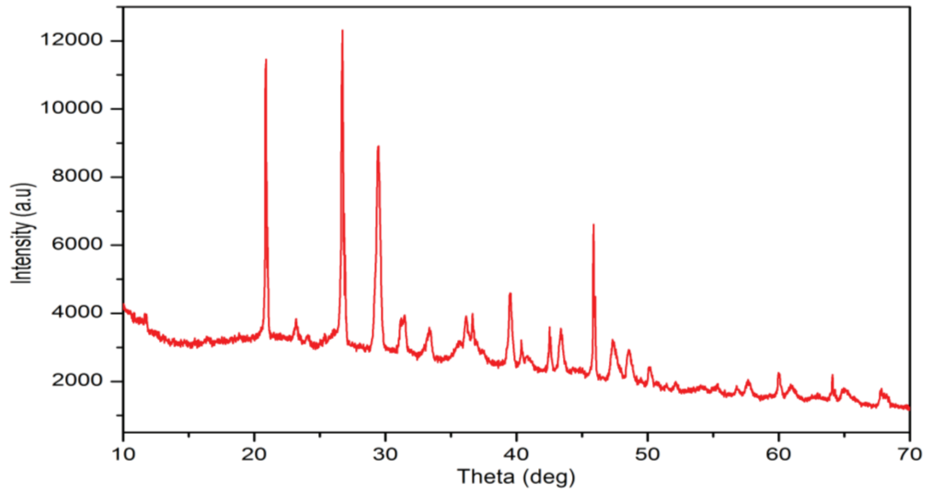

Fig. 6: XRD pattern of the vermicompost.

initial and final stage of ash waste vermicompost samples are shown in Fig. 5. By comparing the initial and final stage there is a strong hydrogen bond due to -OH stretch was observed at 3431.78 cm^{-1}. The band at 1123.67 cm^{-1} and 1117.69 cm^{-1} absorbed for lignin were also observed. While comparing the initial and final stage of vermicompost produced there is a slight reduction observed in the previously discussed band. This proves the partial mineralization of cellulosic material.

Hence the result confirms the reduction of aromatic structure, polypeptides and polysaccharides in the vermicompost prepared using ash waste.

X-ray diffraction analysis: The vermicompost prepared was subjected to XRD analysis and the results are shown in Fig. 6.

The crystallographic nature of the vermicompost prepared was studied using X-Ray Diffractometer (Mohan et al. 2009), Fig. 6. The XRD results indicate that the Quartz peak at 60.9 θ, Mullite peak at 40 θ and Magnetite peak at 31 θ present in the fly ash waste.

CONCLUSION

The waste selected for the study namely fly ash was degraded when it was subjected for vermicomposting along with cow dung in different ratios. The EC, TOM, TOC content has decreased when compared with the initial values. The amount of phosphorus and potassium has increased in the final vermicompost, 1:4 (fly ash + cow dung). Hence, this study confirms the possibility of converting fly ash waste into manure utilizing earthworms and microorganisms.

The role of bacterial species is also highlighted in terms of their enzyme activity and the Genomic DNA helps to identify the bacterial community in the vermicompost which could be utilized for further research. Also, the FTIR results show that the reduction of both aliphatic and aromatic structures in the vermicompost which is associated with extensive organic matter mineralization and from the XRD analysis, Quartz, Mullite, Magnetite waste types of fly ash was identified. It is also understood as in the stress environment, reactive oxygen species (ROS) get potentially increased which could result in changes in the antioxidant enzymes like Catalyses (CAT), Superoxide dismutase (SOD), Glutathione peroxide (GPX) and Glutathione S transferase (GST). The increase and decreased level of ROS can produce oxidative damage to macromolecules such as proteins and nucleic acid due to the stress produced by the presence of foreign material during waste degradation.

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