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Nutrient changes and biodynamics of epigeic earthworms *Eisenia fetida* (Savigny) and *Eudrilus eugeniae* (Kinberg) during recycling of bagasse fly ash

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ABSTRACT

Bioconversion of industrial wastes into nutrient rich product using earthworms is of double interest: on the one hand, a waste is converted into organic fertilizer for soil application and on the other, it controls accumulation of harmful waste that is a consequence of growing industrialization. This study reports the feasibility of utilization of vermicomposting technology for nutrient recovery from sugar industrial waste bagasse fly ash (BFA) mixed with cow dung (CD) in laboratory scale experiment. Two different earthworm species Eisenia fetida and Eudrilus eugeniae were utilized for bioconversion of BFA as well as the quality of the end product. Four different treatments including one control were used for the experiment. Results reveal that significant reduction in total organic carbon (TOC), C:N ratio but increase in total nitrogen (TN), total phosphorus (TP), total potassium (TK), calcium (Ca) and magnesium (Mg) after 60 days of processing in T_2 and T_3 treatments for both species of worms.. E. fetida and E. eugeniae showed maximum biomass production, maximum cocoon numbers and hatchlings production in 1:1 ratio of BFA+CD (T_2) mixture as compared to other treatments and control (BFA alone). Based on investigations it is concluded that vermicomposting using earthworms E. fetida and E. eugeniae could be an alternative technology for the management of BFA if it is amended in 1:1 ratio with cow dung.

Key words: bagasse fly ash, nutrient changes, vermicomposting, *Eisenia fetida, Eudrilus eugeniae*, reproduction.

INTRODUCTION

India is one of the leading growers of sugarcane with an estimated production of approximately 300 million tons in the marketing year 2010-11. Sugar-distillery complexes, integrating the production of cane sugar and ethanol, constitute one of the key agrobased industries. There are presently nearly 500 sugar factories in the country along with around 300 molasses based alcohol distilleries [1]. Enormous amount of solid waste streams generated during sugar manufacturing process including sugarcane trash, bagasse, pressmud and bagasse fly ash. Bagasse fly ash is the waste generated by the combustion of bagasse. Apart from silica which is the major component, it contains other metal oxides as well as unburned carbon [2]. Around 0.005-0.066 tons fly ash is generated per ton during sugarcane crushed [3]. This waste is usually disposed off in pits; it is also applied on land for soil amendment in some areas. Roughly 0.97 million tones of unburned carbon is available from bagasse fly ash alone in India. Disposal of this waste is appropriate one of the major areas of concern for a developing country like India. At present, a very meager quantity of the bagasse fly ash is usually used as fertilizer source and soil conditioner. On the other hand, this approach is not desirable practice in view of the odor from biological degradation. Available literature has proved that application of un-decomposed wastes or non-stabilized compost to land may lead to immobilization of plant nutrients and cause phytotoxicity [4].

Numerous technologies are harnessed to deal with the organics that have the feasible to pollute the environment. Existing technologies concentrate to oxidize the organics in the waste producing a new stream that has its own disposal problems. The need of the hour is to develop close loop technologies which harness the renewable energy and/or nutrient of these waste organics to fuel/or amend the soil. Vermicomposting is a suitable technology to handle different types of organic and industrial solid wastes and make valuable manure from it. Several epigeics (*Eisenia fetida, Eisenia andrei, Eudrilus eugeniae, Perionyx excavatus* and *Perionyx sansibaricus*) have been recognized as potential candidates to decompose organic waste materials [5-7]. With this background, in the present study were performed to investigate the role of *Eisenia fetida* and *Eudrilus eugeniae* in vermicomposting of bagasse fly ash amended with cow dung. So in this study, attempt is being done to investigate the role of *E. fetida* and *E. eugeniae* in bioconversion of bagasse fly ash amended with cow dung and its utilization into natural fertilizer.

MATERIALS AND METHODS

Earthworm cultures: Two composting species of earthworms (*Eisenia fetida* and *Eudrilus eugeniae*) were chosen for the experiment. *E. fetida,* being most commercially used worm for vermicomposting and *E. eugeniae* is a fast-growing and productive earthworm in animal waste that is ideally suited as a source of animal feed protein as well as for rapid organic waste conversion. Hence, both worms were cultured in the laboratory, department of zoology, Annamalai University, Tamilnadu, India and were randomly picked for experimentation.

Experimental design: The bagasse fly ash (BFA) was collected from E.I.D. sugar factory in Nellikuppam, Tamil Nadu, India. The cow dung (CD) was obtained from a dairy farm in Faculty of Agriculture, Annamalai University. The BFA was mixed with CD in different proportions. Four different combinations of BFA and CD were prepared (Table 1). Plastic troughs measuring 30cm height and 30cm diameter were used. Each treatment consisted of three replicates with 2kg of feed materials for both species of worms. The troughs were kept under shade and irrigated with equal quantity of tap water on alternate days to ensure that the substrate moisture content was maintained at approximately 70%. After the completion of pre-inoculation period of 7 days, earthworm species was introduced at 40g per treatment (20g/kg of waste) into all the treatments. Biomass, cocoon numbers and hatchlings production in each treatment was measured at the end of experiment by hand sorting. The sampling of the substrate was done at 0, 15, 30, 45 and 60 days at a depth of 8cm. Substrate samples drawn from all the treatment combinations were dried under shade and nutrient contents were analyzed.

Nutrient analysis: Total organic carbon (TOC) was measured using the method of Walkley and Black [8], total nitrogen (TN) by micro Kjeldahl digestion [9] and total phosphorus (TP) using molybdenum blue method of Olsen et al, 1954 [10]. Total potassium (TK), calcium (Ca) and magnesium (Mg) were measured by a Perkin Elmer 2380 Atomic Absorption Spectrophotometer and DR-3000 Spectrophotometer (HACH). The sample (1g) was digested with a mixture of nitric, sulphuric and precholric acid (3:1.5:2 by volume) at 100°C. The solution was filtered through Whatman filter paper (No.40) for further estimation. C: N ratio was calculated from the measured value of C and N.

RESULTS AND DISCUSSION

A total of four treatments filled with different ratios of BFA mixed with CD were maintained for this study (Table 1). The growth and reproduction of *E.eugeniae* and *E. fetida* was monitored for 60 days. Maximum growth was recorded in T_2 treatment. Similarly maximum cocoons and hatchlings production were also recorded in T_2 treatment, however decreasing proportions of organic supplements (CD) with BFA and BFA alone in the treatment (T_1 and T_4) affected the growth and reproduction of both worms (Tables 2,3). In the present study, both the worms showed maximum and minimum mean individual biomass achieved at end on T_2 and T_3 treatments, respectively. The BFA alone treatment did not show biomass production during vermicomposting; might be due to the substrate quality. Maximum biomass in the treatments may be due to the more palatability and acceptability of feed by earthworms and the minimum biomass in the treatments (T_1 and control) with higher proportion of BFA/ BFA alone was possibly due to the presence of some growth-retarding substances in it. The difference in growth rate among different treatments seems to be closely related to substrate quality [11]. The excellent growth rate of all the earthworms in the treatments containing BFA and CD could be due to its palatability and more acceptability as food by earthworms. The results clearly suggested that importance of bulking material in vermicomposting of BFA and may be justified in terms of the physical, chemical and biological nature of the bulking materials [12].

S.No	Treatments	Composition[a]	
		E. fetida	*E. eugeniae*
1	T_1	BFA + CD (3:1)	BFA + CD (3:1)
2	T_2	BFA + CD (1:1)	BFA + CD (1:1)
3	T_3	BFA + CD (1:3)	BFA + CD (1:3)
4	T_4	BFA alone (control)	

[a] dry weight basis, BFA-Bagasse fly ash; CD-Cow dung

Table 2. Growth and reproduction of *Eudrilus eugeniae* during vermicomposting of bagasse fly ash mixed with cow dung after 60 days

Treatments	Mean initial biomass of individual earthworm(mg)	Maximum individual biomass achieved(mg)	Total number of cocoons at the end	Total number of hatchlings
T_1	320.19±20.1	867.25±28.5	147.21±11.5	82.13±8.0
T_2	320.21±7.5	987.29±32.3	195.15±7.3	171.11±7.2
T_3	319.14±9.2	962.37±18.5	172.29±5.2	143.16±7.6
T_4	319.21±13.5	NA	NA	NA

Results are the mean of three replicates ± standard deviation; Not available.

Table 3. Growth and reproduction of *Eisenia fetida* during vermicomposting of bagasse fly ash mixed with cow dung after 60 days

Treatments	Mean initial biomass of individual earthworm(mg)	Maximum individual biomass achieved(mg)	Total number of cocoons at the end	Total number of hatchlings
T_1	261.3±4.3	619.3±19.4	124.6±12.3	105.6±21.5
T_2	268.3±10.4	795.5±19.5	189.8±15.7	146.5±23.5
T_3	260.6± 8.2	713.2±21.4	171.5±23.4	127.3±18.6
T_4	269.3±6.5	NA	NA	NA

Results are the mean of three replicates ± standard deviation; Not available.

Table 4: Changes in total organic carbon (TOC) content (%) during vermicomposting of bagasse fly ash

Treatments		TOC (%)				
		0 days	15 days	30 days	45 days	60 days
T_1	*E. eugeniae*	45.5±2.19	40.2±2.27	36.5±1.15	28.6±2.35	25.1±1.11
	E. fetida	45.5±2.19	41.7±2.18	37.4±1.49	28.9±2.18	25.8±1.51
T_2	*E. eugeniae*	49.6±1.32	43.7±2.39	33.4±1.25	24.5±2.21	23.3±1.19
	E. fetida	49.6±1.32	43.8±1.85	35.8±1.53	25.6±1.82	24.2±1.39
T_3	*E. eugeniae*	51.3±1.22	41.6±2.41	31.8±2.22	26.2±2.33	24.7±1.31
	E. fetida	51.3±1.22	43.3±2.18	34.1±2.11	26.9±1.67	25.3±1.56
T_4 (Control)		40.2±1.21	35.1±1.17	29.3±1.26	27.5±1.41	26.6±1.18

Results are the mean of three replicates ± standard deviation

Table 5: Changes in total Kjeldahl nitrogen (TN) content (%) during vermicomposting of bagasse fly ash

Treatments		TN (%)				
		0days	15 days	30 days	45 days	60 days
T_1	*E. eugeniae*	1.17±0.21	1.66±0.47	1.93±0.25	2.10±0.23	2.12±0.21
	E. fetida	1.17±0.21	1.52±0.34	1.88±0.55	2.04±0.19	2.04±0.35
T_2	*E. eugeniae*	1.29±0.35	1.93±0.56	2.40±0.42	2.42±0.47	2.30±0.27
	E. fetida	1.29±0.35	1.82±0.19	2.37±0.28	2.38±0.41	2.25±0.29
T_3	*E. eugeniae*	1.42±0.27	1.87±0.31	2.31±0.19	2.38±0.19	2.31±0.18
	E. fetida	1.42±0.27	1.80±0.29	2.26±0.49	2.29±0.59	2.30±0.38
T_4 (Control)		1.11±0.15	1.58±0.51	1.72±0.27	1.91±0.36	2.01±0.17

Results are the mean of three replicates ± standard deviation

Table 6: Changes in C:N ratio during vermicomposting of bagasse fly ash vermicomposting of bagasse fly ash

Treatments		C:N ratio				
		0days	15 days	30 days	45 days	60 days
T_1	*E. eugeniae*	38.89±1.24	24.22±1.67	18.91±1.72	13.64±0.23	11.84±0.41
	E. fetida	38.89±1.24	27.43±1.58	19.94±1.65	14.17±0.37	12.65±0.30
T_2	*E. eugeniae*	38.45±1.55	22.64±1.82	13.92±1.92	10.12±0.34	10.13±0.19
	E. fetida	38.45±1.55	24.07±1.69	15.11±1.85	10.76±0.26	10.76±0.22
T_3	*E. eugeniae*	36.13±1.49	22.25±1.45	13.77±1.21	11.01±0.51	10.51±0.27
	E. fetida	36.13±1.49	24.06±1.51	15.09±1.55	11.75±0.47	11.00±0.45
T_4 (Control)		36.22±1.61	22.20±2.04	17.30±1.38	14.40±0.99	13.23±0.34

Results are the mean of three replicates ± standard deviation

Table 7: Changes in total phosphorus (TP) content (%) during vermicomposting of bagasse fly ash vermicomposting of bagasse fly ash

Treatments		TP (%)				
		0days	15 days	30 days	45 days	60 days
T_1	*E. eugeniae*	1.02±0.25	1.07±0.21	1.08±0.29	1.13±0.31	1.12±0.33
	E. fetida	1.02±0.25	1.04±0.38	1.11±0.36	1.12±0.24	1.10±0.46
T_2	*E. eugeniae*	1.21±0.24	1.27±0.15	1.29±0.52	1.32±0.18	1.36±0.29
	E. fetida	1.21±0.24	1.21±0.26	1.24±0.31	1.30±0.47	1.30±0.42
T_3	*E. eugeniae*	1.20±0.18	1.25±0.44	1.27±0.63	1.30±0.25	1.35±0.61
	E. fetida	1.20±0.18	1.25±0.52	1.27±0.18	1.29±0.41	1.33±0.34
T_4 (Control)		1.01±0.15	0.99±0.38	1.05±0.13	1.09±0.21	1.15±0.15

Results are the mean of three replicates ± standard deviation

Table 8: Changes in total potassium (TK) content (%) during vermicomposting of bagasse fly ash vermicomposting of bagasse fly ash

Treatments		TK (%)				
		0days	15 days	30 days	45 days	60 days
T_1	*E. eugeniae*	1.52±0.15	1.61±0.19	1.75±0.26	1.77±0.13	1.79±0.42
	E. fetida	1.48±0.24	1.55±0.32	1.71±0.13	1.76±0.59	1.79±0.36
T_2	*E. eugeniae*	1.71±0.19	1.79±0.20	1.82±0.17	1.85±0.24	1.80±0.21
	E. fetida	1.65±0.32	1.68±0.38	1.77±0.13	1.79±0.28	1.81±0.12
T_3	*E. eugeniae*	1.78±0.15	1.81±0.24	1.87±0.25	1.89±0.38	1.86±0.17
	E. fetida	1.70±0.21	1.73±0.47	1.80±0.39	1.83±0.41	1.85±0.25
T_4 (Control)		1.21±0.42	1.54±0.44	1.53±0.12	1.52±0.33	1.50±0.11

Results are the mean of three replicates ± standard deviation

Table 9: Changes in total calcium (Ca) content (%) during vermicomposting of bagasse fly ash vermicomposting of bagasse fly ash

Treatments		Ca (%)				
		0days	15 days	30 days	45 days	60 days
T_1	*E. eugeniae*	1.21±0.21	1.25±0.18	1.29±0.12	1.38±0.21	1.51±0.24
	E. fetida	1.21±0.27	1.25±0.23	1.28±0.18	1.35±0.14	1.48±0.32
T_2	*E. eugeniae*	1.29±0.57	1.29±0.37	1.37±0.36	1.45±0.27	1.59±0.57
	E. fetida	1.29±0.34	1.29±0.14	1.35±0.19	1.40±0.33	1.54±0.37
T_3	*E. eugeniae*	1.32±0.47	1.35±0.25	1.39±0.31	1.46±0.25	1.61±0.31
	E. fetida	1.32±0.31	1.34±0.36	1.35±0.27	1.42±0.33	1.57±0.25
T_4 (Control)		1.10±0.30	1.13±0.19	1.22±0.35	1.26±0.19	1.29±0.18

Results are the mean of three replicates ± standard deviation

Table 10: Changes in total magnesium (Mg) content (%) during vermicomposting of bagasse fly ash vermicomposting of bagasse fly ash

Treatments		Mg (%)				
		0days	15 days	30 days	45 days	60 days
T_1	*E. eugeniae*	0.75±0.05	0.79±0.05	0.82±0.02	0.89±0.05	0.87±0.08
	E. fetida	0.75±0.08	0.76±0.02	0.84±0.06	0.87±0.06	0.88±0.05
T_2	*E. eugeniae*	0.71±0.04	0.75±0.03	0.82±0.05	0.87±0.08	0.85±0.07
	E. fetida	0.71±0.05	0.74±0.09	0.81±0.07	0.89±0.08	0.84±0.04
T_3	*E. eugeniae*	0.63±0.05	0.67±0.05	0.75±0.02	0.82±0.04	0.80±0.09
	E. fetida	0.63±0.09	0.64±0.05	0.78±0.05	0.84±0.05	0.82±0.05
T_4 (Control)		0.40±0.07	0.83±0.05	0.85±0.04	0.83±0.02	0.81±0.05

Results are the mean of three replicates ± standard deviation

Cocoon production patterns of earthworms during vermicomposting were directly related to the quality of feedstock. Earthworms showed better reproduction performances in bedding containing appropriate or acceptable ratio of bulking materials [11]. Earlier studies advocate the importance of greater nitrogen fractions in enhanced cocoon production rates in epigeics [13]. The difference between rates of cocoon production in different feed mixtures could be related to the biochemical quality of the substrate, which is one of the important factors in determining onset of cocoon production [14]. In the present study maximum number of cocoon production was observed in T_2 and T_3 and minimum was recorded in T_1 treatment for both species of worms. On the other hand cocoon production was not found in BFA alone treatment it may be due to absence of bulking materials in the treatments.

Hence, the results suggested that higher proportions of BFA / BFA alone in the treatments were not suitable for cocoon production. It may be concluded that production of cocoons in the feed mixtures could be related to the biochemical quality of the feed, which was one of the important factors and in addition to the biochemical properties of waste, the microbial biomass and decomposition activities during vermicomposting are also important in determining the cocoon production [15]. Similarly the maximum number of hatchlings was observed in T_2 and T_3 than the other treatments for both the earthworm species (Tables 2, 3). Hatchlings production was higher in treatments, which contained equal proportions of BFA and bulking material (CD). Monroy *et al.* [16] reported that production of cocoons and hatchlings depends upon the densities of the earthworms and feed material in the treatment. Hence, in the present study, it may be concluded that difference in hatchlings production in the treatments may be due to stocking concentration and environmental conditions [17] during bioconversion of BFA.

The total organic carbon (TOC) decreased in all the treatments (T_1, T_2 and T_3) including control (T_4) after vermicomposting (Table 4). Periodical study recorded a variable change in TOC content during vermicomposting of different treatments for both worms. However reduction in TOC was comparatively higher in T_2 and T_3 treatments than T_1 treatment and control (T_4). The loss in TOC during vermicomposting earthworms promoted such microclimatic conditions in the treatments that increased the loss of TOC from substrates through microbial respiration. In the present study, total nitrogen (TN) content of the substrates increased progressively during the development of decomposition in all treatments except control (Table 5). The increase in TN content of the organic waste during decomposition is well recognized [18]. Mineralization of organic N to inorganic N during decomposition could have attributed to the increase of N content in the amendments. On the other hand, TN content of T_2 treatment remained stable during the decomposition process which may be due to the low C:N ratio of BFA that favors microbial flare up. The decrease in C:N ratio was rapid up to day 45, thereafter, it showed a more or less stabilized pattern up to day 60 (Table 6). In the present study, decline of C:N ratio to less than 20 after day 30 indicates an advanced degree of organic matter stabilization and reflects a satisfactory degree of maturity of organic wastes [19].

The TP content nearly doubled in treatment that had a combination of BFA and CD under 1:3 and 1:1 ratios during a period of 60 days decomposition for *E. eugeniae* and *E. fetida*. Increase in TP during vermicomposting is probably through mineralization and mobilization of phosphorus by bacterial and phosphatase activity of earthworms [20]. The TK content was also greater in all the vermicompost than contol at the end of experiment (Table 7). The maximum increase in TK was higher in T_2 and T_3 as compared to the T_4 (control) treatment at the end of experiment for both species of worms (Table 8). According to Barois and Lavelle [21] earthworm primes it's symbiotic gut microflora with secreted mucus and water to increase their degradation of ingested organic matter and the release of assailable metabolites. Therefore, directly or indirectly earthworm enriches the substrate material with exchangeable-K. Ca content of treatments containing BFA and CD at different proportions increased steadily during the composting process (Table 9). Mg content of vermicompost in T_2 and T_3 treatments slightly increases during 30–60 days decomposition (Table 10). This is obvious that the substrate blended with cow dung increased the feeding ability of the both worms which favorably enhanced the Ca and Mg content of the vermicompost during decomposition of BFA [22]. Ca content of treatments (T_1, T_2 and T_3) containing BFA and CD at different proportions decreased steadily during the experimentation.

CONCLUSION

This is evident that the substrates blended with BFA and CD increased the feeding ability of the earthworms *E. fetida* and *E. eugeniae* which favorably enhanced the nutrient contents of the substrate during decomposition. Nevertheless, Nutrient changes during vermicomposting did not show significant difference among T_2 and T_3 treatments. The vermicompost obtained from T_2 and T_3 at 45 days were rich in important plant nutrients (nitrogen, phosphorus and potassium) and their C:N ratio was below 20 which indicated their agronomic importance. Hence this study indicated that BFA amendments up to 50% with CD may help in economic utilization in vermicomposting. In addition, *E. fetida* and *E. eugeniae* appeared to modify the degrading activity of the substrate to a much greater level than the sole use of BFA in vermicomposting. This was reflected by the lower C:N ratio, as well as by a gradual release of nutrients in this study which made the vermicompost from BFA more appropriate substrates for agronomic purpose.

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