GROWTH AND REPRODUCTIVE PERFORMANCE OF *EISENIA FOETIDA* IN COW MANURE, COW MANURE + SUGARCANE BAGASSE, AND COW MANURE + SAWDUST WASTE

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Abstract. Accumulation of sawdust and sugarcane bagasse can cause serious problems for the environment. In this study, we examined the growth and reproductive performance of *Eisenia foetida* in cow manure, cow manure + sugarcane bagasse (4:1, V/V) and cow manure + sawdust (4:1, V/V) waste and its effects on CO_2 evolution. The results showed that the weight gain for *E. foetida* (live weight) per gram of dry weight of the feed source in cow manure + sugarcane bagasse (39 ± 0.66 mg/g) and cow manure waste (37 ± 0.36 mg/g) were greater than in cow manure + sawdust waste (34 ± 1.05 mg/g). The number of cocoons produced per earthworm per day in different wastes was in the order: cow manure + sugarcane bagasse > cow manure + sawdust > cow manure. After adding earthworms to cow manure, cow manure + sugarcane bagasse, and cow manure + sawdust, CO_2 emissions rapidly decreased after 15 days. Statistical analysis showed that after 90 days, vermicompost wasn't significantly different (p = 0.05) from compost in terms of production of nitrogen, phosphorus, potassium and pH level. Our experiments presented vermicomposting using *E. foetida* as an alternative technology that can be used to recycle S and SB in laboratory conditions.
Keywords: *earthworms, cocoon, waste, growth, CO_2, microbial biomass, physicochemical characteristics*

Introduction

Over the last few years, the interest in using earthworms as an ecological system for manure management has tremendously increased. Various researchers have tested earthworm-processed wastes, usually called vermicompost in the horticulture and agriculture industries (Atiyeh et al., 2004; Arancon et al., 2005; Azizi, et al., 2008). During vermicomposting process, the important plant nutrients, such as nitrogen, potassium, phosphorous and calcium present in feed material are converted into much more soluble and available to plants than those in the parent compounds (Ndegwa and Thompson, 2001). Because of the uniformity of its composition, vermicompost reduces

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pollutants and tends to hold more nutrients over a longer period without any impact on the environment and is considered an excellent product.

The potential of *Perionyx excavates* in vermicomposting of different wastes, such as sheep dung, cow dung, biogas sludge, and poultry manure, was reported by Kale et al. (1982). Loh et al. (2004) reported higher cocoon production and weight gain by *E. foetida* in cattle waste than in goat waste. Gunadi and Edwards (2003) have studied growth, reproduction, and mortality of *E. foetida* for over a year in solid manure, pig manure, and supermarket waste solids. Worms could not survive in fresh cattle solids, pig solids, fruit wastes, or vegetable wastes. The growth of *E. foetida* in pig wastes was faster than in cattle solids. After 60 weeks of the experiment, several times of adding the substrate increased reproduction of worms, but worms showed a tendency to lose weight. Organic matter, microorganisms, and plants are some components of the environment that continually influence agricultural systems (Silvana et al., 2001).

During vermicomposting, earthworms eat and grind substrates, convening with anaerobic micro flora to increase the surface area for microbial colonization and enzymatic action (Edwards and Fletcher, l988). *E. foetida* is an epigeic earthworm species that lives in organic wastes and requires high moisture content, adequate amounts of suitable organic material, and dark conditions for proper growth and development (Gunadi and Edwards, 2003; Gunadi et al., 2002).

Throughout the world, earthworms play an important role in determining the balance of greenhouse gases from soils, and their impact is expected to increase in the coming decades (Lubbers et al., 2013). Respiratory CO2 (a measure of metabolic activities) and the enzymes involved in the various chemical changes in the soil are usually used as the index in some laboratory studies in which manure in incubators is modified over several weeks by the addition of earthworms. It showed that earthworms greatly reduce respiration and soil enzyme activities (Pichtel and Hayes, l990).

This paper aims to investigate the effect of CM, CM + SB, and CM +S waste on the life cycle of *E. foetida* and its effects on CO2 evolution. It was hypothesized that S and SB waste would affect the life cycle of *E. foetida* due to differences in physico-chemical characteristics.

Material and Methods

Three treatment groups were studied in triplicate, containing a mixture of cow manure (CM) alone, cow manure (CM) + sugarcane bagasse (SB), and cow manure (CM) + sawdust (S), all with a ratio of 4: 1 (V/V), respectively.

Growth, sexual development, and cocoon production

Nine one liter plastic containers (Three treatments in the three replicates) (diameter 12 cm, depth 10 cm) were filled with 150 g (air dry) of CM + EW (earthworms), CM + SB + EW, and CM + S + EW. To eliminate the escape of toxic gases, wastes were manually stirred daily over a period of 15 days. After fifteen days, seven non-clitlellated hatchlings of *E. foetida*, weighing 200–250 mg (live weight), were placed into a container. The moisture content of the waste was kept within the range of 80–70% humidity throughout the study period. Growth chamber had an internal stabilization system for temperature and humidity, but to make sure of enough moisture, distilled water was sprayed. All containers were kept in a dark growth chamber at 25 ± 1°C (*Figure 3*). Weight gain, clitellum development, and cocoon production were recorded

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weekly for 12 weeks (*Figure 4*). The feed was removed from the container, and earthworms and cocoons were separated from the feed by hand sorting. After counting them, the development clitellum was examined and weighed after being washed with water and dried with toilet paper. The worms were weighed without removing their gut contents. For any data obtained in this study, correction for gut contents was not used. Then, all earthworm food (but no cocoons) was returned to the container. No food was added at any stage during the study period. The cocoons viability was determined weekly for 12 weeks by removing them from the plastic containers and putting them in a Petri dish filled with distilled water. All petri dishes were kept in darkness at a temperature of 25 ± 1°C. To prevent bacterial growth and avoid negative impacts on the results, the water in these dishes was replaced daily. Cocoons and hatchlings for each cocoon were recorded over a period of 12 weeks.

CO2 evolution

Eighteen one-liter plastic containers (diameter 12 cm, depth 10 cm) were filled with 150 g (dry air) of CM, CM + SB, CM + S, CM + EW (earthworms), CM + SB + EW, or CM + S + EW (Six treatments in the three replicates). The moisture content of wastes was adjusted in the range of 70 –80% during the study period by spraying adequate quantities of distilled water. All containers were kept in darkness at a temperature 25 ± 1°C (*Figure 3*). Waste was manually stirred daily for 15 days in order to eliminate the escape of toxic gases. The compost (treatments without earthworms) was produced parallel to vermicompost (treatments with earthworms). After the 15 days, seven non-clitlellated hatchling of *E. foetida*, weighing 200–250 milligrams (live weight), were placed in each vermicompost container. Samples were taken at 0, 15, 30, 45, 75, and 90 days. Day 0 refers to the initial mixing of the waste before preliminary decomposition. Earthworms collected by hand sorting and the wastes without earthworms were evaluated for CO_2 evolution. The alkali trap method was used to quantify the released CO_2 (Anderson, 1982). Conical respiration flasks (500 mL) contained the earthworm treatments and assimilation vials that each contained 10 mL of 0.3 M NaOH. Flasks containing the alkali traps alone served as controls. The Alkali traps were replaced in each of the samples, and were titrated with 0.1 M HCl (Anderson, 1982). The evolved CO_2 obtained from the titration data was corrected with control data. All experiments were performed in triplicate, and the results were averaged.

Physicochemical analysis

The pH was determined in a double distilled water suspension of each mixture with a ratio of 1:5 (W/V) that had been shaken mechanically for 30 min and filtered through a whatman No.1 filter. The electrical conductivity of this solution was measured by a conductivity meter (Verdonck and Gabriels, 1992). For all samples, total nitrogen was determined by the Bremner and Mulvaney (1982) procedure after digesting the sample with concentrated H2SO4 and HClO4 (9:1, V/V). Each sample (2 g) was ashed in a muffle furnace at 550°C for determination of other nutrients (Horwitz, 1980). The white ash was then dissolved in 2N HCl, and distilled water was added to bring the total volume to 100 mL. Total P was measured with spectrophotometer by using the Murphy and Riley (1962) procedure with molybdenum in sulfuric acid. Total K was measured with a flame photometer using the Houba et al., (1989) method, after digesting the

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sample in the diacid mixture (concentrated HNO_3: $HClO_4$, 4:1, V/V). The Nelson and Sommer (1982) method was used to measure total organic carbon.

Statistical analyses

The data were analyzed by SAS and means were compared by Tokay's multiple range tests at 5% probability level (SAS Institute, 2001).

Results

The primary physicochemical properties by CM, SB, S, CM+S and CM+SB waste are summarized in *Table 1*. The waste moisture varied between 14.40% and 15.20%. The highest electrical conductivity was in SB waste (3.19 µS/cm) and the lowest was in S waste (0.46 µS/cm). The OC ranged from 23.24% in CM+SB waste to 57.18% in S waste. The C/N ratio was the lowest in CM+SB waste and the highest in S waste, 12.16 and 181.81, respectively. The potassium content ranged from of 0.40% in SB waste to 1.11% in CM+S waste. Phosphorus ranged from 0.04% in SB waste to 0.56% in CM+SB waste.

Table 1. *First physicochemical characteristics of CM, SB and S waste.*

Waste	Moisture content (%)	pH (1:5)	Conductivity (µS/cm)	OC (%)	N (%)	C/N ratio	K (%)	P (%)
CM	14.40	8.15	2.06	47.30	1.24	38.12	0.62	0.29
S	14.85	7.37	0.46	57.18	0.31	181.81	0.61	0.34
SB	15.05	7.79	3.19	50.23	0.45	111.75	0.40	0.04
CM+S	15.14	7.20	2.00	23.62	1.73	13.68	1.11	0.78
CM+SB	15.20	8.47	2.99	23.24	1.91	12.16	0.86	0.56

CM, cow manure; SB, sugarcane bagasse; S, sawdust; OC: Organic Carbon

Growth of E. foetida in the wastes

No mortality was observed in any waste during the study period. In this experiment, all waste was stirred by hand daily for 15 days to prevent the accumulation of toxic gas. The growth rate of *E. foetida* in investigating waste during the study period is given in *Figure 1*.

Maximum worm weight was obtained in CM + SB waste (621 ± 119 mg/earthworm) and minimum in CM + S (557 ± 490 mg/earthworm). Maximum earthworm weight was obtained in the 7th, 9th, and 12th week, for CM, CM + SB, and CM + S waste, respectively. Initially, the earthworms gained weight, but after a few weeks, earthworm weight loss was observed in all waste groups. This earthworm weight loss can be attributed to the depletion of food. The highest weight per g dry weight of food for *E. foetida* was obtained in CM + SB waste (39±0.66 mg/g) and the lowest in CM + S (34 ± 1.05 mg/g) waste. The weight gain in our study was only 37 ± 0.66 mg/g by *E. foetida* species in CM at 25 °C.

Net weight gain/earthworm per unit of food material in different feeds will follow this order: CM + SB > CM > CM + S. Similarly, we found that net weight gain by earthworms in CM + SB waste was 1.1 times higher than in CM + S waste (*Table 2*).

Accordingly, the highest growth rate was obtained in waste CM + SB (6.9 ±1.18) and the lowest growth rate in CM + S (6.1±1.96) (*Table 2*).

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Table 2. *Growth of Eisenia foetida in CM, SB and S waste.*

Treatments	Mean first weight/ earthworm (mg)	Maximum weight reached /worm(mg)	Maximum weight reached on	Net weight gain/worm (mg)	Growth rate /worm/day (mg)	Worm weight each unit dry waste (mg/g)
CM	234±25	618±123	7th week	557±490	6.6±0.61	37±0.36
CM+S	228±16	557±490	12th week	513±166	6.1±1.96	34±1.05
CM+SB	208±70	621±119	9th week	579±100	6.9±1.18	39±0.66

CM, cow manure; SB, sugarcane bagasse; S, sawdust; All values was introduced as the mean ± SD (standard deviation).

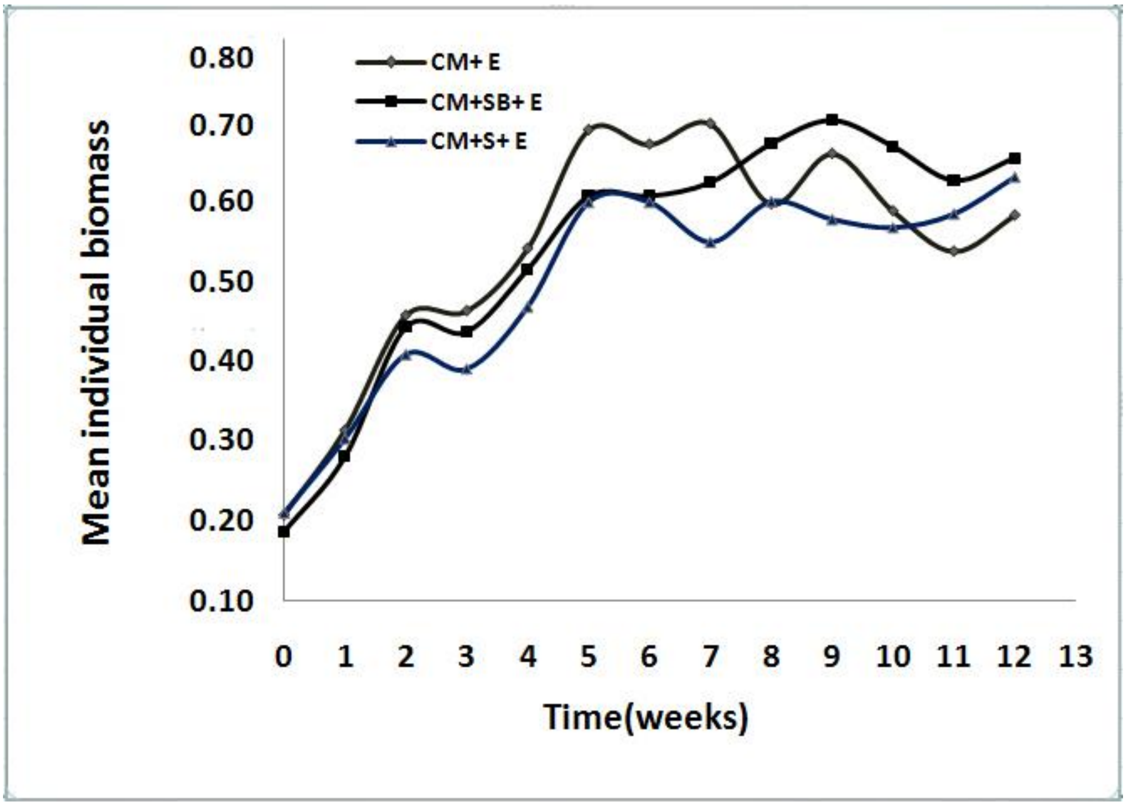

Figure 1. *The Eisenia foetida growth on different wastes. Abbreviations: CM, cow manure; SB, sugarcane bagasse; S, sawdust; E, earthworms*

Sexual development and cocoon production

Table 3 summarizes the sexual development and cocoon production of earthworms in various feeds. Individual clitellum developed by day 28 in all food types. Cocoon production of earthworms in all waste was started about 35 days. Figure 2 shows the cumulative cocoon production by earthworms in different feeds. After 12 weeks maximum cocoons were found in CM + SB waste (215 ± 30) and minimum in CM waste (191 ± 20).

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Table 3. *Cocoon production by Eisenia foetida in CM, SB and S waste.*

Treatments	Clitellum development started in	Cocoon production started in	Total no. of cocoons produced after 12 weeks	No. of cocoons produced/ worm	No. of cocoons produced/ worm /day	Cocoon production stopped after
CM	3th week	5th week	191±20	30.2±1.70	0.36±0.02	12th week
CM+S	4th week	5th week	209±46	30.8±4.26	0.37±0.05	12th week
CM+SB	4th week	5th week	215±30	31.5±6.61	0.38±0.08	12th week

CM, cow manure; SB, sugarcane bagasse; S, sawdust;
All values was introduced as the mean ± SD(standard deviation).

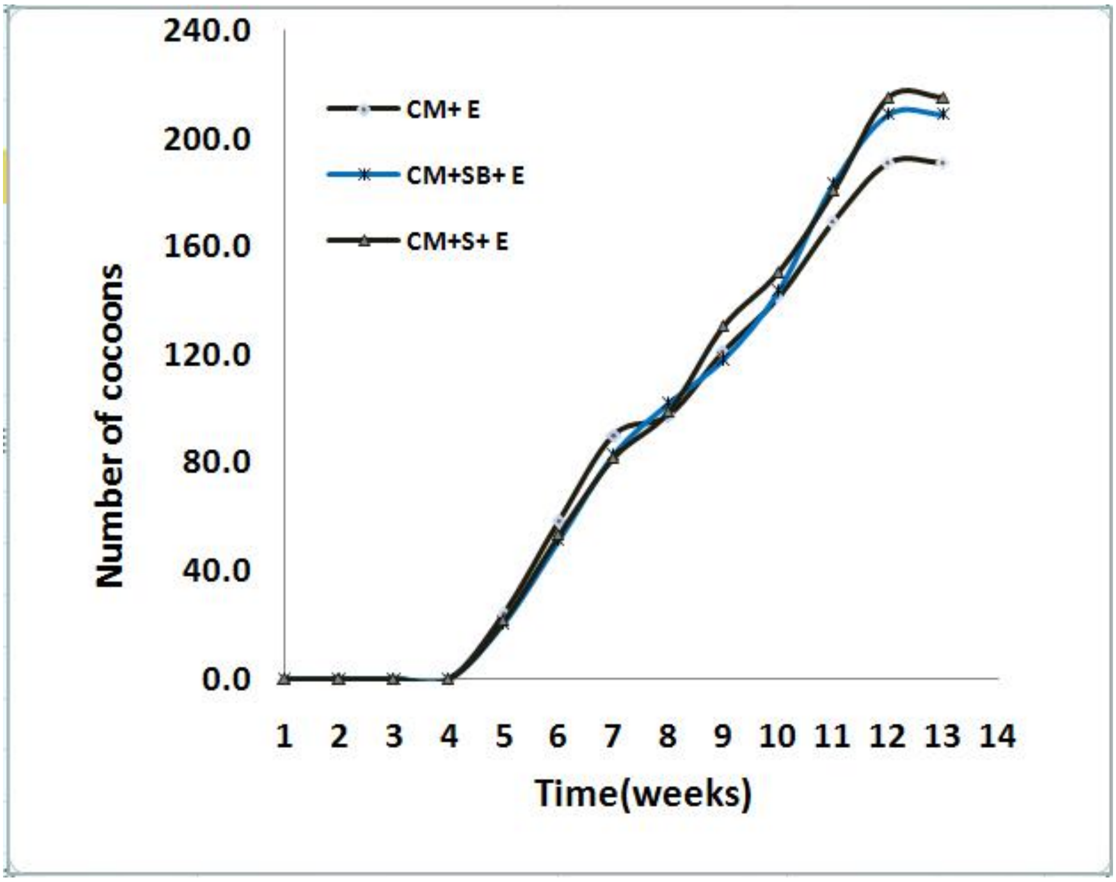

Figure 2. *Cumulative cocoon production by Eisenia foetida on different wastes. Abbreviations:CM, cow manure; SB, sugarcane bagasse; S, sawdust; E, earthworms*

Cocoon production of earthworms per day in different waste will follow this order: CM + SB > CM + S > CM (Table 4). We suggest that CM + S and CM + SB waste are good foods for supporting weight and the reproduction of earthworms.

Table 4 summarizes the viability of *E. foetida* cocoon in CM, SB, and S waste. The maximum number of hatchlings was produced after 12 weeks in CM waste (428 ± 27) and the minimum in CM + SB waste (393 ± 77). Mature worms spend a lot of energy

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producing their cocoons. Cocoon production was stopped by day 84 in all waste conditions.

Table 4. *Cocoon viability in CM, SB and S waste.*

Treatments	Total no. of hatching produced after 12 weeks	No. of hatching produced/ cocoons
CM	428±27	2.3±0.30
CM+S	439±54	2.1±0.43
CM+SB	393±77	1.9±0.15

CM, cow manure; SB, sugarcane bagasse; S, sawdust;
All values were introduced as the mean ± SD (standard deviation).

Figure 3. *Growth chamber for keeping containers.*

Figure 4. *The cocoons of Eisenia foetida and hatchling emerged from them.*

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Table 5 shows the results of changes in CO2 evolution during composting of CM, SB and S waste in the absence and presence of earthworms in a 90-day period. Respiration rate in the studied waste rapidly decreased after 15 days (*Table 5*). The ratio of N, P, K and pH in compost inoculated with earthworms wasn't different from compost without worms (*Table 6*).

***Table 5**. Change in CO_2 evolution (µg CO_2 g^{-1} h^{-1}) during composting of organic wastes*

Treatments	Days					
	0	15	30	45	75	90
CM	30.4 a	11.7 bc	8.4 bd	5.0 bd	5.2 bd	3.8 cd
CM+ EW	30.4 a	8.6 bd	9.1 bd	4.0 cd	3.4 cd	2.4 d
CM+S	26.5 a	10.1 bd	7.1 bd	5.8 bd	4.5 bd	4.2 bd
CM+S+ EW	26.5 a	11.2 bc	7.8 bd	6.9 bd	6.0 bd	4.6 bd
CM+SB	28.6 a	12.5 b	7.2 bd	4.5 bd	5.2 bd	4.2 bd
CM+SB+ EW	28.6 a	23.8 a	8.1 bd	6.1 bd	5.3 bd	5.2 bd

Abbreviations: CM, cow manure; SB, sugarcane bagasse; S, sawdust.
Means followed by the same letters do not significantly differ (p = 0.05).

***Table 6.** Physicochemical properties of treatments after 90 days of composting*

Treatments	Total N (%)	Total P (%)	Total K (%)	PH (1:5)
CM	1.75 a	0.48 ab	1.01 ac	8.11 ab
CM+ E	1.81 a	0.50 a	1.13 ab	8.00 b
CM+S	1.44 ab	0.38 bc	0.88 bd	7.30 c
CM+S+ E	1.47 ab	0.39 b	1.15 a	7.20 c
M+SB	1.53 a	0.46 ab	0.74 df	8.46 a
CM+SB+ E	1.67 a	0.46 ab	0.82 ce	8.18 ab

Abbreviations: CM, cow manure; SB, sugarcane bagasse; S, sawdust; E, earthworms
Means followed by the same letters do not significantly differ (p = 0.05).

Discussion

Gunadi and Edwards (2003) reported mortality of *E. foetida* after two weeks in fresh manure even though factors such as pH, electrical conductivity, the ratio of C: N, and NH4 + and NO3 contents were suitable for the growth of earthworms. They believed that the earthworm death were due to anaerobic conditions that had been generated after two weeks in the fresh cow manure. It is acknowledged that to prevent mortality of earthworms, pre-composting is essential.

Neuhauser et al. (1980) reported that the rate of weight gain by *E. foetida* is dependent on population density and the type of food. According to Edwards et al. (1998), growth rate is a good indicator for comparing the growth of earthworms in different wastes. The weight gain for *E. foetida* (live weight) per gram dry weight of the feed of CM + SB (39 ± 0.66 mg/g) and CM (37±0.36 mg/g) wastes was more than that of CM + S wastes (34 ± 1.05 mg/g). Edwards et al., (1998) reported generation of 292 mg/g weight by *P. excavatus* in CM at 25 °C, but in our study was only 37 ± 0.66 mg/g by *E. foetida* species. This difference could be due to difference in species morphology or primary characteristics of the feed wastes.

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Cocoon production rates may be related to biochemical differences between the quality of feeds, which are important in determining the time to reach sexual maturity and begin reproduction (Edwards, 1988; Edwards et al., 1998). Cocoons production per earthworm in each day of treatment observed the following pattern from the highest to the lowest: CM + SB > CM + S > CM. These wastes can support growth and reproduction of *E. foetida*, and thus can be used as foods in a large scale vermicomposting facility. Feeds which can provide enough easily metabolizable organic matter and carbohydrates are favorable for earthworm growth and reproduction (Edwards, 1988). When cocoons are not being produced, energy is used for tissue growth (Chaudhari and Bhattacharjee, 2002; Kale et al., 1982). Satchell (1967) showed that the effects of earthworms in readily degradable organic substances, which contain a high microbial population, are not significant in comparison with soil.

Another parameter that represents the stabilization rate in manure containing earthworms is respiration rate (i.e., CO_2 production) (Atiyeh et al., 2000). In the first four weeks of the experiment, microbial activity and earthworm activity progressed. The rapid decrease in the rate of evolution of CO_2 from the CM, CM + S, and CM + SB wastes showed that many biodegradable materials were being rapidly destroyed. Two weeks after the addition of earthworms to waste, CO2 evolution rapidly decreased and continued to more slowly decrease until week 12, which indicates increased stability in organic matter. Similarly, Bautista et al. (2011) reported that more than 70% of the total CO_2 evolution occurs during the first week of the composting process. Generally, activities of earthworms in the soil, enhances the number and biomass of microbes (Edwards and Bohlen, 1996). Zhang et al. (2000) reported that earthworms use micro-organisms as a secondary food source; total soil microbial biomass reduced transit time through the earthworm gut, while active components of microbial biomass increased transit time. The results correspond with the results of Grapelli et al. (1983), who noted that earthworms selectively support the microorganisms that are responsible for the transformation of organic matter in the soil. This can be attributed to the effect of earthworms on organic acid accumulation resulting from microbial metabolism, or breakdown of folic and humic acid (Albanell et al., 1988; Chen and Griffiths, 1988). Similar results on vermicomposting of cow manure, fruit, and vegetable wastes have been reported by Azizi et al. (2008), Gunadi and Edwards (2003) and Mitchell (1997).

Conclusions

This study results indicate the potential for vermicomposting of S or SB wastes in combination with cow manure and using earthworm *E. foetida* as an alternative technology for the recycling of this type of waste. Further studies for investigating the possibility of using S and SB in combination with other livestock wastes are required.

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ELECTRONIC APPENDIX

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