Vermicomposting of Sewage Sludge: Multiple Effects of Bulking Materials and Dry Solids Content on the Growth and Reproduction of the Earthworm *Eisenia foetida* (A Pilot Scale Study)

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The multiple effects of residual bulking agents type (paper, saw dust, straw) in mixtures with activated sludge and dry solids content (D.S., 10, 15 and 20 %) in 10 treatments on growth and reproduction of *Eisenia foetida* was studied in pilot scale experiments with batches of 50 earthworms for 10 weeks period. The maximum weight achieved was attained in the mixture of sludge with saw dust, dry solid 20 % (772 ± 47 mg d^{-1}) and the highest growth rate was attained in the mixture of sludge with paper, dry solid 15 % (13.5 ± 1.41 mg d^{-1}).The highest reproduction rate was observed in the mixture of sludge with paper, dry solid 15 % (2.5 ± 0.12 cocoon $earthworm^{-1}$ $week^{-1}$) compared to the lowest in the control with activated sludge alone (0.09 ± 0.02 cocoon $earthworm^{-1}$ $week^{-1}$).

Key Words: *Eisenia foetida*, Earthworms, Sewage activated sludge, Bulking agents, Growth rate, Reproduction.

INTRODUCTION

Studies about use of earthworms to stabilization of sewage sludge began about 3 decade ago[1]. It was demonstrated quite early, at a laboratory scale, that aerobic sewage sludge can be decomposed and stabilized by *Eisenia foetida* about 3 time as fast as non-earthworm conditions because of the increases in rates of microbial decomposition in the casts of earthworms. Most of the sludge produced in sewage treatment plants is anaerobic and when fresh, can be toxic for earthworms, but after becoming aerobic, it is easily accepted and ingested by them. There are many basic aspects about vermicomposting of sewage sludge those have not been well studied and need to be researched, evaluated and resolved to assure success of this process.

Mixing sewage sludge with other materials such as paper, saw dust and straw or other carbon-rich wastes and composting the mixture using earthworms, can accelerate decomposition, due to maceration and mixing of such materials during passage through the earthworm gut and passage into earthworm casts. These bulking

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materials can also improve C:N ratio by supplying C and at the same time prevent N losses by ammonia volatilization[2].

It is well known that the food source influence not only the size of earthworms population but also their growth and reproduction rates. There is abundant literature on the response of earthworms to different types of vegetable substrates in the field[3,4], but there is a lack of information about the effects of diet on earthworms during the vermicomposting process[5].

In this paper, the multiple effects of some organic bulking agents (paper, saw dust and straw) mixed with sewage activated sludge and dry solid content (10, 15 and 20 %) have been investigated in a pilot scale trial on growth and reproduction of *Eisenia foetida* and therefore on the stabilization of sludge. This study is about other and further aspects of the trial was accomplished by Dominguez *et al.*[5].

EXPERIMENTAL

Young non-clitellated specimens of *Eeisenia foetida* weighing 170-220 mg were randomly picked up from several cow manure stock culture of earthworm *Eisenia foetida* from vermicomposting unit of Tabriz municipality in Iran. Sewage aerobic activated sludge biosolids were obtained from a wastewater treatment plant in Tabriz. Each batches of 50 earthworms were placed in each of 36 liters capacity plastic containers (length 28 cm, width 22 cm, depth 10 cm) with proper drainage system and supplied 3.5 kg of a mixture of sewage sludge with bulking agents. Ten treatments (each treatment in 3 replicates) were considered in this research, include the mixture of sludge with paper in 3 treatment of dry solids (D.S.) *i.e.*, D.S. 10, 15 and 20 %; the mixture of sludge with saw dust in 3 treatment (D.S. 10, 15 and 20 %); the mixture of sludge with straw in 3 treatment (D.S. 10, 15 and 20 %); and alone sludge used as blank. The weight of earthworms, clitellum developments and cocoon production (determined by hand-sorting) and volatile solids (V.S.) content of treatments were measured weekly for 10 weeks and during this period no additional feed of mixtures was supplied. To compare the growth rates of *Eisenia foetida* in the different mixtures, mg weight gained day^{-1} $worm^{-1}$ was used as a common denominator for comparison.

Chemical analyses of mixtures: A subsample (*ca.* 50 g) of each materials and mixtures was dried at 105 °C and was ashed at 550 °C to determine dry solids and organic matter (volatile solids) content, respectively. The pH and conductivity were determined using water diluted samples (1:10) by electrometry method and NH_3-N using macro kjeldal method.

Statistical analyses: One-way ANOVA based on least significant differences (LSD ≤ 0.05) and two-way ANOVA are applied to determination of significant differences in different mixtures about growth and reproduction of earthworms. The principal component analysis (PCA) was carried out using correlation matrix of biological parameters (maximum weight, growth rate, maturation time and cocoon production).

RESULTS AND DISCUSSION

The sewage activated sludge utilized in this experiment showed low ammonia content (1690 µg g^{-1}) and conductivity value (2000 µS cm^{-1}) which are adequate for rearing *Eisenia foetida* and consequently earthworm survival was high (92 %) in the control. During study (10 weeks) average of minimum and maximum temperature were 14.49 ± 0.24 and 21.09 ± 0.38 °C, respectively and overall average temperature was 17.79 ± 0.30 °C. Earthworm mortality was low (< 6 %) in all the mixtures except in one with straw (D.S. 10 %) which was near to 16 % of individuals died during two first weeks.

The growth curves of *Eisenia foetida* in sewage activated sludge and in the mixtures with different bulking materials and dry solids content are given in Figs. 1 and 2. The earthworms reached their maximum weights in the sludge mixture with saw dust (D.S. 20 %) among all treatments and in alone sludge among treatments with the same bulking agents, named main treatments (772 ± 47 mg and 767 ± 42 mg, respectively). The smallest size was attained in the mixture with straw (D.S. 10 %) among all treatments and in with straw among main treatments (545 ± 27 mg and 634 ± 42 mg, respectively). The highest growth rate was observed in the mixture with paper (D.S. 15 %) among all treatments and in alone sludge among main treatments (13.50 ± 1.41 and 13.22 ± 0.99 mg day^{-1} $worm^{-1}$) and the lowest was attained in the mixture with straw (D.S. 10 %) among all treatments and in the mixture with straw among main treatments (7.21 ± 0.70 and 8.90 ± 0.90 mg day^{-1} $worm^{-1}$, respectively). Tables 1-3 show earthworms growth results. The maximum weight was achieved sooner in the mixtures with paper and alone sludge (6th week) than mixtures with saw dust and straw (7th week). The highest net weight gain per worm was attained in the mixture with saw dust (D.S. 20 %) among all treatments and in alone sludge among main treatments (584 ± 52 and 555 ± 41 mg, respectively) and the lowest in the mixture with straw (D.S. 10 %) among all treatments and in mixture with straw among main treatments (353 ± 34 and 436 ± 44 mg, respectively).

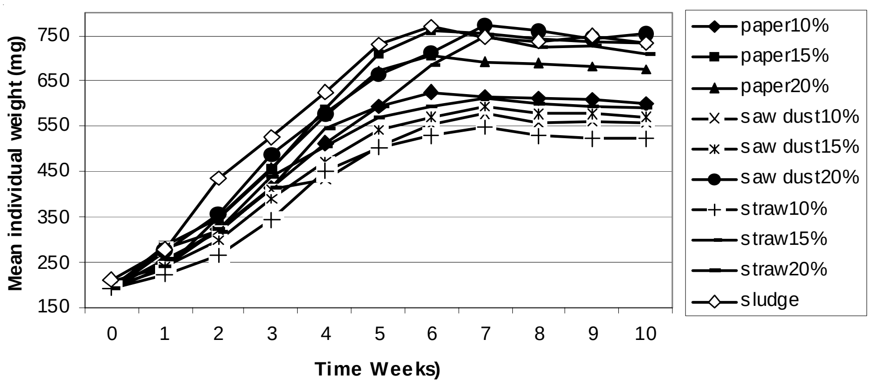

Fig. 1. Growth of *Eisenia foetida* in different diet mixtures (all treatments)

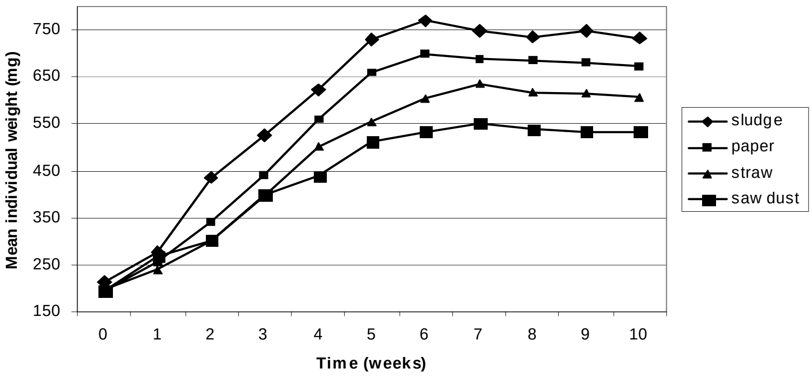

Fig. 2. Growth of *Eisenia foetida* in different diet mixtures (main treatments)

TABLE-1
GROWTH OF *Eisenia foetida* IN DIFFERENT DIET MIXTURES (ALL TREATMENTS)

Treatment	Mean initial weight per earthworm (mg)	Maximum weight achieved per earthworm (mg)	Maximum weight achieved on (i-th week)	Net weight gain per earthworm (mg)	Growth rate per worm per day (mg/earth-worm/day)
Sludge + Paper (D.S. 20 %)	193.57±5.74	701±40 abcd	6th	507±45 abc	12.07±1.07 a
Sludge + Paper (D.S. 15 %)	190.57±4.53	758±59 ac	6th	567±59 a	13.50±1.41 a
Sludge + Paper (D.S. 10 %)	198.07±4.00 [1]	622±40 abcd	6th	424±39 abc	10.09±0.93 abc
Sludge + Straw (D.S.20%)	209.03±2.09	610±62 bcd	7th	401±64 bc	8.18±1.31 bc
Sludge + Straw (D.S.15%)	191.73±6.10	746±77 acd	7th	554±83 ac	11.31±1.69 ac
Sludge + Straw (D.S. 10%)	192.00±8.43	545±27 b	7th	353±34 b	7.21±0.70 b
Sludge + Saw dust (D.S. 20 %)	188.70±4.66	772±47 a	7th	584±52 a [2]	11.91±1.06 a
Sludge + Saw dust (D.S. 15 %)	190.00±6.35	590±38 bd	7th	400±45 bc	8.17±0.91 bc
Sludge + Saw dust (D.S.10 %)	195.06±2.83	577±75 b	7th	382±77 b	7.79±1.56 bc
Sludge	212.16±0.96	767±42 a	6th	555±41 ac	13.22±0.99 a

1: Mean ± SE (Standard error).

2: Treatments followed by at least one same letter are not significantly different ($p \leq 0.05$).

The highest mean maturation size was observed in mixture with saw dust (D.S. 20 %) among all treatments and in alone sludge among main treatments (664 ± 34 and 623 ± 37 mg, respectively) and the lowest in mixture with straw (D.S. 10 %), among all treatments and in mixture with paper, among main treatments (500 ± 35 and 555 ± 19 mg, respectively).

TABLE-2
GROWTH OF *Eisenia foetida* IN DIFFERENT DIET MIXTURES (MAIN TREATMENTS)

Treatment	Mean initial weight per earthworm (mg)	Maximum weight achieved per earthworm (mg)	Maximum weight achieved on (i-th week)	Net weight gain per earthworm (mg)	Growth rate per worm per day (mg/earth-worm/day)
Sludge + Paper	194.07±2.64[1]	693±31 [a]	6th	499±32 [a]	11.89±0.76[a]
Sludge + Straw	197.61±4.20	634±42 [a]	7th	436±44 [a]	8.90±0.90[c 2]
Sludge + Saw dust	191.25±2.61	646±42 [a]	7th	455±44 [a]	9.29±0.89[bc]
Sludge	212.16±0.96	767±42 [a]	6th	555±41	13.22±0.99[a]

1: Mean ± SE (Standard error).
2: Treatments followed by at least one same letter are not significantly different ($p \leq 0.05$).

TABLE-3
GROWTH OF *Eisenia foetida* IN DIFFERENT DIET MIXTURES
(BASED ON D.S. % CONTENT)

Treatment	Mean initial weight per earthworm (mg)	Maximum weight achieved per earthworm (mg)	Maximum weight achieved on (i-th week)	Net weight gain per earthworm (mg)	Growth rate per worm per day (mg/earth-worm/day)
Dry solids 20 %	197.10±3.78[1]	694±34 [ab]	6.67±0.17	497±38 [a]	10.72±0.86[ab]
Dry solids 15 %	190.79±2.88	698±40 [ab 2]	6.67±0.17	507±42 [a]	10.99±1.03[a]
Dry solids 10 %	195.04±2.95	581±28 [bc]	6.67±0.17	386±29 [b]	8.36±0.72[b]
Sludge	212.16±0.96	767±42 [a]	6	555±41 [a]	13.22±0.99[a]

1: Mean ± SE (Standard error).
2: Treatments followed by at least one same letter are not significantly different ($p \leq 0.05$).

Figs. 3 and 4 show the total cocoon production in alone sewage sludge and in the mixtures with the different bulking agents. Cocoon production was significantly higher in the mixtures of sewage sludge with paper (2.17 ± 0.14 cocoons week^{-1} earthworm^{-1}) than in the other mixtures. The lowest cocoon production was achieved in the alone sewage sludge (0.09 cocoons week^{-1} earthworm^{-1}). Tables 4-6 show achieved results about reproduction of earthworms.

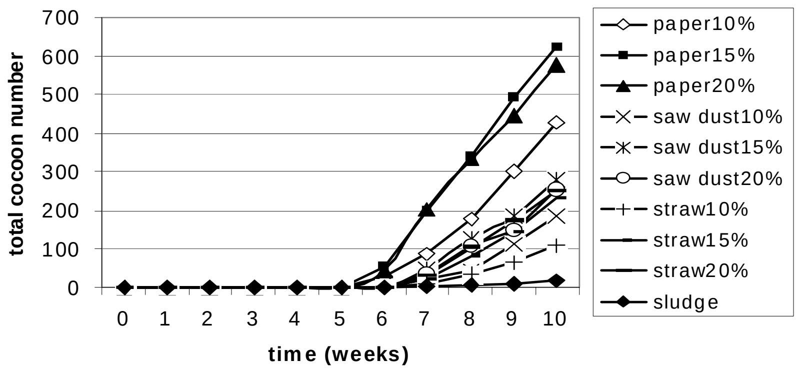

Fig. 3. Reproduction of *Eisenia foetida* in different diet mixtures (all treatments)

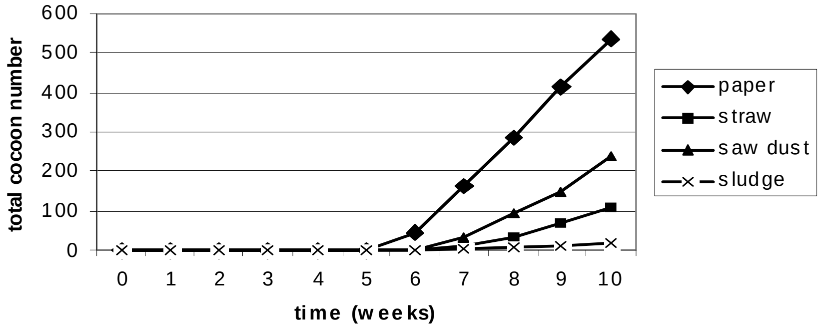

Fig. 4. Reproduction of *Eisenia foetida* in different diet mixtures (main treatments)

TABLE-4
REPRODUCTION OF *Eisenia foetida* IN DIFFERENT DIET MIXTURES (ALL TREATMENTS)

Treatment	Clitellum development started in (i-th week)	Cocoon production started in (i-th week	Total no. of cocoon produced after 10 weeks	No. of cocoons produced per earthworm	No. of cocoons produced per earthworm/ week	Mean maturation size (mg)
Sludge + Paper (D.S. 20 %)	4	6	576±31 [ac 1]	11.51±0.61[a]	2.30±0.12 [b]	572±28[abc]
Sludge + Paper (D.S. 15 %)	4	6	625±30 [a]	12.50±0.60[a]	2.50±0.12[a]	584±34 [abc]
Sludge + Paper (D.S. 10 %)	4	6	426±49 [dc]	8.53±0.97[b]	1.71±0.19[c]	509±27[b]
Sludge + Straw (D.S. 20 %)	5	7	251±37 [ef 2]	5.03±0.74[ed]	1.26±0.18[gh]	566±54 [abc]
Sludge + Straw (D.S. 15 %)	5	7	232±9 [ef]	4.65±0.18 [ed]	1.16±0.04[h]	598±27 [abc]
Sludge + Straw (D.S. 10 %)	5	7	109±13 [g]	2.19±0.27 [f]	0.54±0.07[e]	500±35[b]
Sludge + Saw dust (% D.S. 20)	5	7	253±47 [ef]	5.06±0.93 [ed]	1.27±0.23[gh]	664±34[a]
Sludge + Saw dust (% D.S.15)	5	7	281±11 [e]	5.61±0.22 [e]	1.40±0.05[g]	541±14[bc]
Sludge + Saw dust (D.S. 10 %)	5	7	184±23 [fg]	3.68±0.46[df]	0.92±0.12[d]	505±33[b]
Sludge	4	7	18±4 [b]	0.36±0.08 [c]	0.09±0.02[f]	623±37[ac]

1: Mean ± SE (Standard error).
2: Treatments followed by at least one same letter are not significantly different ($p \leq 0.05$).

Two-way ANOVA (Table-7) showed that: (1) Type of bulking materials was more effective than D.S. content on growth and reproduction of earthworms; (2) Effects of bulking agents type and D.S. content on reproduction were higher than growth of earthworms; (3) There were no interactive effects between type of bulking materials and D.S. content on growth and reproduction of earthworms.

TABLE-5
REPRODUCTION OF *Eisenia foetida* IN DIFFERENT DIET MIXTURES (MAIN TREATMENTS)

Treatment	Clitellum development started in (i-th week)	Cocoon production started in (i-th week	Total no. of cocoon produced after 10 weeks	No. of cocoons produced per earthworm	No. of cocoons produced/ earthworm per week	Mean maturation size (mg)
Sludge + Paper	4	6	542±33 [a 1]	10.85±0.70[a]	2.17±0.14[a]	555±19 [a]
Sludge + Straw	5	7	198±25 [bc 2]	3.95±0.5 [c]	0.99±0.13[bc]	555±25 [a]
Sludge + Saw dust	5	7	239±21 [c]	4.78±0.42[bc]	1.20±0.11[c]	570±28 [a]
Sludge	4	7	18±4 [d]	0.36±0.08[d]	0.09±0.02[d]	623±37 [a]

1: Mean ± SE (Standard error).
2: Treatments followed by at least one same letter are not significantly different ($p \leq 0.05$).

TABLE-6
REPRODUCTION OF *Eisenia foetida* IN DIFFERENT DIET MIXTURES (BASED ON D.S. % CONTENT)

Treatment	Clitellum development started in (i-th week)	Cocoon production started in (i-th week	Total no. of cocoon produced after 10 weeks	No. of cocoons produced per earthworm	No. of cocoons produced/ earthworm per week	Mean maturation size (mg)
Dry solids 20 %	4.67±0.17 [1]	6.67±0.17	360±57 [a]	7.20±1.15 [a]	1.61±0.2 [ab]	601±26 [a]
Dry solids 15 %	4.67±0.17	6.67±0.17	379±63[a]	7.59±1.25 [a]	1.69±0.21 [b]	574±16 [a]
Dry solids 10 %	4.67±0.17	6.67±0.17	240±50 [ab]	4.80±1.01[ab]	1.06±0.18 [c]	505±16 [b]
Sludge	4	7	18±4 [b 2]	0.36±0.08[bc]	0.09±0.02 [d]	623±37 [a]

1: Mean ± SE (Standard error).
2: Treatments followed by at least one same letter are not significantly different ($p \leq 0.05$).

TABLE-7
DETERMINATION OF SIGNIFICANT DIFFERENCE AMONG DIFFERENT BULKING AGENTS AND D.S. % BY TWO-WAY ANOVA

Two-way ANOVA			
Variable	Significant difference among ($p \leq 0.05$)		
	Different bulking agents (paper, straw, saw dust)	Different D.S. % (20%, 15%, 10%)	Interaction
Growth rate per earthworm per day	s. ($p \leq 0.05$)	s. ($p \leq 0.025$)	n.s.
Max. weight achieved per earthworm	s. ($p \leq 0.05$)	n. s.	n.s.
Mean maturation size	s. ($p \leq 0.01$)	n. s.	n.s.
Net weight gain per earthworm	s. ($p \leq 0.05$)	n. s.	n.s.
No. of cocoons produced/earthworm per week	s. ($p \leq 0.01$)	s. ($p \leq 0.01$)	n.s.
Total cocoon number	s. ($p \leq 0.01$)	s. ($p \leq 0.01$)	n.s.
Total cocoon produced/earthworm	s. ($p \leq 0.01$)	s. ($p \leq 0.01$)	n.s.

s. = Significant difference n.s. = Non-significant difference.

Principal component analysis (Table-8) revealed the presence of two main factors accounting for 85.5 % of variance. The first (principal component-1) of the principal component analysis explains 59.70 % of environmental variance, which represents the growth of *Eisenia foetida* in the different mixtures and is good and negatively correlated with the growth rate (-0.623, Table-8). The second (principal component-2) of principal component analysis accounts for 25.8 % of environmental variance and it was defined as the effect of reproduction, explained by cocoon production that is high correlated with principal component-2 (0.762, Table-8).

TABLE-8
FACTOR LOADING OF THE PRINCIPAL COMPONENT ANALYSIS

Factor	Growth (principal component-1)	Reproduction (principal component-2)
Growth rate	-0.623	-0.258
Maximum weight	-0.565	-0.438
Cocoon production	-0.297	0.762
Maturation time	0.452	-0.402
Eigenvalue	2.388	1.031
Variance	0.597	0.258

This study showed again that the bulking agent had a much important effect on the reproduction than on the growth of *Eisenia foetida*[5] and among all treatments, only sludge + paper (D.S. 15 %) had the maximum growth rate and cocoon production at the same time; but among main treatments, the maximum growth rate and cocoon production were not observed in the same mixtures[5].

Comparing present results with others in literature shows that earthworms have higher growth rate in the sludge and in the mixture of sludge with paper, pointing out the high potential of sewage sludge for vermicomposting[6].

Present results about growth rate, 7.21-13.50 mg day^{-1} earthworm^{-1}, with respect to the scale of study (pilot scale) are similar to others in literature, 8.60-11.43 mg day^{-1} worm^{-1} for *E. foetida*[7]; 7-19 mg day^{-1} worm^{-1} for *E. foetida*[8]; 14 mg for *E. foetida*[9]; 4.5 mg for *E. andrei*[10]; 7 mg for *E. foetida*[11]; 12.25-16.75 mg for *E. andrei*[12]; 15-18.6 mg for *E. andrei*[5].

This study confirm the general rule, also reported in the literature so that the minimum weight for maturation is *ca.* 0.4 g[5,13-15]. The minimum weight for maturation in this study was 0.500-0.623 g.

Present results about cocoon production, 0.92-2.50 cocoons week^{-1} earthworm^{-1} in mixtures of sludge with bulking agents and 0.09 cocoons week^{-1} earthworm^{-1} in sludge alone, with respect to study scale are similar to others in the literature, 3.8 cocoons week^{-1} earthworm^{-1} for *E. foetida*[6]; 3.08 cocoons week^{-1} earthworm^{-1} for *E. andrei*[16]; 0.08-3.19 cocoons week^{-1} earthworm^{-1} in mixtures of sludge with bulking agents and 0.05 cocoons week^{-1} earthworm^{-1} in sludge alone[5].

The fact that the control (alone sludge) showed a very low reproductive rate indicates that sewage sludge is much better substrate for vermicomposting when mixed with other bulking materials.

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