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BIOCONVERSION OF SOLID PAPER-PULP MILL SLUDGE BY EARTHWORMS

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Abstract
*Bioconversion of solid paper-pulp mill sludges and primary sewage sludge for 40 days at a ratio of 3:1 dw:dw was studied in containers with and without earthworms (*Eisenia andrei*). This mixture was a suitable medium for optimum growth and reproduction of the earthworms. Regardless of the presence of earthworms, degradation occurred during the bioconversion period, but the presence of earthworms accelerated the mineralization of organic matter, favored the breakdown of structural polysaccharides and increased the humification rate. Consequently, the C/N ratio and the degree of extractability of heavy metals were lower in the worm-worked end product. Copyright © 1996 Elsevier Science Ltd.*

Key words: Earthworms (*Eisenia andrei*), growth, chemical composition, carbohydrates, organic matter, solid paper-pulp sludge, primary sewage sludge, bioconversion.

INTRODUCTION

Earthworms have increasingly been used for organic waste management (animal and plant wastes, municipal garbage, municipal and industrial sludges) in order to obtain a more stable and easily handled material. This material, called vermicompost, worm-cast or worm-worked organic product, has been traditionally used as organic fertilizer, soil amendment and a component of potting media (Handreck, 1986; Edwards & Burrows, 1988).

The sludges resulting from the depuration process of paper-pulp mill wastewater are difficult to eliminate. They are extremely stable due to the slow biodegradability of structural polysaccharides (Adney *et al.*, 1991), their major constituents. Previous studies have shown that these wastes can be reutilized through vermicomposting (Butt, 1993; Elvira *et al.*, 1995). The addition of nitrogen-rich organic wastes seems to be a necessary pretreatment in vermicomposting, in order to provide nutrients and an inoculum of microorganisms. The combined actions of the microorganisms with the detritivorous activity of earthworms (*Eisenia andrei*) then promotes bioconversion.

This study examines the efficiency of an epigeic earthworm *Eisenia andrei* (Bouché) in bioconverting solid paper-pulp mill sludge mixed with primary sewage sludge. The earthworm growth and reproductivity were evaluated and compared with the changes in chemical composition of this mixture in the presence and absence of earthworms.

METHODS

Preparation of the waste

Fresh solid paper-pulp mill sludges (PMS) were obtained from the wastewater treatment plant of a sulfate-paper-pulp factory (Empresa Nacional de Celulosas, S.A.) located in Pontevedra, Spain. The main characteristics of this sludge are reported in Table 1. This sludge was mixed with primary sewage sludge [total solids: 77 g kg^{-1}, pH: 5.3, total organic carbon (TOC): 325 g kg^{-1}, total Kjeldahl nitrogen (TKN): 18.5 g kg^{-1} and C/N: 17.6] obtained from a wastewater treatment plant in Santiago de Compostela, Spain, at a ratio of 3:1 (3 parts PMS:1 part

Table 1. Characteristics of solid paper-pulp mill sludge

Total solids, g kg^{-1}	189
pH	9.2
TOC, g kg^{-1}	541
TKN, g kg^{-1}	2
C:N	270
Total carbohydrates, g kg^{-1}	981
TFA soluble carbohydrates, g kg^{-1}	77
TFA non soluble carbohydrates, g kg^{-1}	904

All data, except total solids and pH, are expressed on dry matter basis.

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primary sewage sludge; dw:dw). This mixture was turned over manually every 48 h for a period of 10 days in order to eliminate volatile substances potentially toxic to earthworms. After this period, a homogenized sample of this mixture (initial mixture, IM) was air-dried at room temperature, ground in a stainless-steel blender and stored in a plastic vial.

Experimental design

Two cylindrical plastic containers (16 cm dia × 25 cm high) were filled with the initial mixture (1200 g dw each). To one cylinder (W) was added 50 non-clitellated earthworms (*Eisenia andrei*) weighing between 0.10–0.20 g. No worms were placed in the other cylinder (NW). Both cylinders were kept in darkness at room temperature (20–25°C). The moisture content of the mixture in each cylinder was kept at 80–85% throughout the bioconversion period. Growth, mortality, clitellum development and cocoon production were measured approximately once a week for 40 days, as follows. The substrate in the cylinder was turned out, and earthworms and cocoons were collected from the whole mixture by hand-sorting, after which they were counted, weighed and examined for sexual condition. Then all measured earthworms and the substrate (but not cocoons) were returned to the cylinder. At every sampling the cylinder without worms was handled similarly in order to assess environmental conditions in both treatments. At the end of the experimental period the earthworms and cocoons were separated and the substrate from each cylinder was air-dried at room temperature. Homogenized samples of both mixtures (W, NW) were ground and stored in plastic vials.

Chemical analysis

The stored samples at the beginning (IM) and at the end (W, NW) of the bioconversion period were analyzed in triplicate, except for carbohydrate determinations. The pH was determined potentiometrically (Crison 2000 pH meter) in distilled water (ratio 1:20). Total organic carbon was measured using the dichromate acid oxidation method according to Nelson and Sommers (1982). Total Kjeldahl nitrogen (TKN) was determined after digestion with concentrated H_2SO_4 according to the Brenmer & Mulvaney (1982) procedure. Total P was analyzed using a calorimetric method with molybdenum in sulfuric acid (Peech *et al.*, 1947). Crude fiber was determined according to the AOAC (1984). Total K, Ca, Fe, Mn, Cu, Zn, Cd, Ni, Pb and Co were determined by means of atomic absorption spectrophotometry (AAS) after digestion of the samples with HNO_3:$HClO_4$ (2:1) (AOAC, 1984). Available heavy metals were determined using AAS after extraction with 0.005 M DTPA—0.01 M $CaCl_2$–0.1 M TEA solution, pH 7.3 (Lindsay & Norwell, 1978).

Organic matter fractions in initial and final products were determined according to Sequi *et al.* (1986). The organic carbon was extracted with 0.1 M $Na_4P_2O_7$, pH < 2, and centrifuged to separate humic acids (HA). Supernatants were fed on to polyvinylpyrrolidone (Aldrich, chromatography-grade cross-linked PVP) and two fractions were obtained: the non-retained (non-humified fraction, NH) and the retained fraction (fulvic acids, FA), which was desorbed with alkaline solutions. Total organic carbon was determined in TEC, NH, HA and FA. Three humification parameters were estimated: the humification index, calculated as HI = NH/[HA+FA] (Sequi *et al.*, 1986), the degree of humification, as DH(%) = ([HA+FA]/TEC) × 100, and the humification rate, as HR(%) = [HA+FA]/TOC) × 100 (Ciavatta *et al.*, 1988).

Total carbohydrates from homogenized samples were analyzed according to Albersheim *et al.* (1967). TFA-non-soluble carbohydrates were determined by hydrolysing with 2 N trifluoracetic acid at 121°C for 1 h. Total carbohydrates were determined after treatment with 72% H_2SO_4 for 3 h at room temperature prior to dilution to 1M acid and heating at 100°C for 150 min. In both cases, total sugar and uronic acid contents in each sample were determined by the phenol–sulfuric acid method (Dubois *et al.*, 1956) and the m-phenyl phenol method (Blumenkrantz & Asboe-Hansen, 1973), respectively.

RESULTS AND DISCUSSION

Growth and reproduction of *Eisenia andrei*

Figure 1 shows the values obtained for different parameters of growth and reproduction in *Eisenia andrei* during the experimental period. Biomass

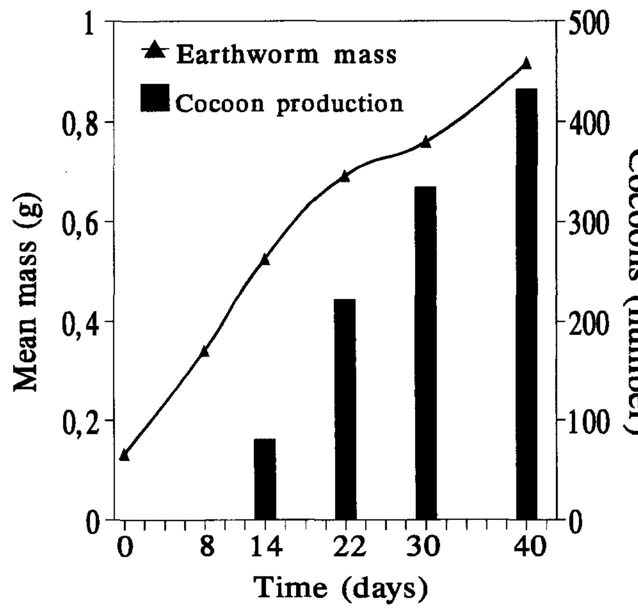

Fig. 1. Growth and cumulative cocoon production of *Eisenia andrei* during the experimental period in the mixture of solid paper-pulp sludge and primary sewage sludge.

increased to a maximum mean weight of 0.90 g; the growth rates corresponding to 0–20 and 20–40 day intervals were 27.14 and 10.52 mg worm^{-1} day^{-1}, respectively. Mortality did not surpass 2%, and all individuals developed the clitellium before day 21 (45% on day 12). Total biomass increased from 6.5 to 44.1 g in 40 days, laying 432 cocoons. These results show that the solid paper-pulp mill sludge amended with primary sewage sludge seems to be a suitable growth medium for earthworms, allowing high rates of reproduction. This mixture probably provides earthworms with easily metabolizeable organic matter characteristic of urban sludges, nutrients, microorganisms and non-assimilated carbohydrates derived from the hydrolysis of carbohydrates, that favor growth and reproductivity of these invertebrates (Flack & Hartenstein, 1984; Edwards & Fletcher, 1988).

Changes in chemical composition

At the end of the bioconversion period, a significant fraction of the TOC contained in the initial mixture was lost as CO_2; this loss was stimulated by the presence of earthworms (Table 2). Similarly, crude fiber was significantly lower at the end of the bioconversion period; this decrease was more evident in the presence of earthworms (76%) than in their absence (66%). The worm-worked product had a higher level of TKN, and consequently a lower C/N ratio, than the non-worm-worked product. In contrast, no significant differences were obseved in K and Ca concentrations.

Total and DTPA-extractable heavy-metal concentrations in the final products were higher than the initial mixture (Table 3). These increases, caused by the mineralization of organic matter, were more evident in the worm-worked material. However, total metal concentrations in both final products were lower than the maximum permissible levels for sludges used for agricultural purposes (Council of the European Commission, 1986). On the contrary, the degree of extractability of metals (percentage DTPA-extractable fraction related to total concentration), except for Mn, decreased during bioconversion. The degree of extractability of all metals was slightly lower in the final worm-worked product than in the final product obtained in the absence of earthworms. Decreased extractability can be explained as a consequence of bioxidation processes themselves, which can cause partial immobilization of metals through the formation of slightly soluble oxides, hydroxides and carbonates, as well as stable complexes combined with the humified organic fraction (García *et al.*, 1990; Cannarutto *et al.*, 1991).

Table 2. The pH, total organic carbon (TOC), nutrient content and crude fiber in the initial mixture (IM) and in the final worm-worked (W) and non-worm-worked (NW) products

		After 40 days	
	IM	W	NW
pH	8.1b	8.8a	8.3b
TOC, g kg^{-1}	424a	247c	296b
TKN, g kg^{-1}	11b	38a	19b
C:N	40a	6.4b	16b
Total P, g kg^{-1}	4.6a	4.2a	4a
Total K, g kg^{-1}	1.2a	1.1a	1a
Total Ca, g kg^{-1}	66a	55a	70a
Crude fiber, g kg^{-1}	523a	124b	178b

All data are expressed on dry matter basis.
Values in the same row followed by the same letter are not significantly different ($P<0.05$).

Table 3. Heavy-metal content (mg kg^{-1}) and degree of extractability (%) of metals in the initial mixture (IM) and in the final worm-worked (W) and non-worm-worked (NW) products. (Cadmium and cobalt concentration in the different organic samples were below the reliable detection limits of the analytical procedure used)

		After 40 days	
	IM	W	NW
Total Fe	2930b	7025a	6145a
DTPA-Fe	481b	719a	787a
Fe extractability	16a	10c	13b
Total Mn	218b	455a	400a
DTPA-Mn	96c	198b	226a
Mn extractability	44b	43b	56a
Total Cu	205b	420a	355c
DTPA-Cu	115a	176a	156a
Cu extractability	56a	42a	44a
Total Zn	370c	795a	680b
DTPA-Zn	309a	345a	312a
Zn extractability	83a	43b	46b
Total Pb	61b	137a	124a
DTPA-Pb	24b	44a	42a
Pb-extractability	39a	32a	34a
Total Ni	20b	54a	44a
DTPA-Ni	6b	13a	13a
Ni extractability	40a	24a	29a

All data are expressed on dry matter basis.
Values in the same row followed by the same letter are not significantly different ($P<0.05$).

Changes in carbohydrate content

The organic matter contained in the solid paper-pulp mill sludge consisted mainly of carbohydrates (981 g kg^{-1} dm), being mostly TFA-non-soluble carbohydrates (e.g. cellulose-type polysaccharides and other strongly bound forms) (Table 1). After the mixture of the sludges was aerated, levels of total carbohydrates of this initial mixture were lower (706 g kg^{-1} dm), although the TFA-soluble fraction (e.g. monosaccharides, disaccharides and non-cellulosic polysaccharides) (Table 4) increased appreciably in comparison with the original paper-pulp mill sludge (Table 1).

As expected, mineralization of organic matter during the bioconversion period notably reduced the total carbohydrate content at the end of the experiment (Table 4). In comparison with the initial mixture, the losses of total carbohydrates were

Table 4. Carbohydrate content (g kg^{-1}) in the initial mixture (IM) and in the final worm-worked (W) and non-worm-worked (NW) products

		After 40 days	
	IM	W	NW
Total carbohydrates	706	137	199
TFA soluble carbohydrates	158	86	124
Neutral sugars	124	61	93
Uronic sugars	34	25	31
TFA non soluble carbohydrates	547	51	75
Neutral sugars	484	44	69
Uronic sugars	63	7	6

All data are expressed on dry matter basis.

higher in the presence of earthworms (80%) than in their absence (72%). The percentage losses were similar to that found for crude fiber. Although this reduction was seen in both TFA-non-soluble and TFA-soluble carbohydrates, the decrease was smaller in the latter fraction. The reduction in TFA-non-soluble carbohydrates was 90.6% in the presence of earthworms and 86.3% without earthworms. In addition, the decreases in TFA-soluble carbohydrates were 45.2% in the presence of earthworms and 21.5% without earthworms. The smaller decrease in the TFA-soluble fraction may be due to the supply in this fraction of single carbohydrates from the hydrolysis of TFA-non-soluble carbohydrates. Neutral sugars and uronic acids in both fractions showed a similar degree of reduction. These results show that earthworms accelerated the hydrolysis of carbohydrates in the substrate, both indirectly by promoting microbial activity (Edwards & Lofty, 1972) and directly through the activity of carbohydrase enzymes (xylanases and cellulases) produced by tissues of the gut wall of the worms (Loquet & Vinceslas, 1987; Whiston & Seal, 1988; Urbasek, 1990; Urbasek & Pizl, 1991). The lower content of TFA-soluble carbohydrates in the worm-worked product indicates that the earthworms may consume these compounds in their diet.

Changes in organic matter fractions

Total extractable carbon (TEC) had increased by the end of the bioconversion period, with larger values appearing in the worm-worked end product (Table 5). These increases were due to the higher levels of C content of the humified (humic acids and fulvic acids) and the non-humified fraction. Both end products had similar levels of humic acid C and non-humified fraction C, whereas the levels of fulvic acid C were significantly higher in the worm-worked end product. Despite these increases, humification index and degree of humification did not show appreciable changes, probably because the relative percentages in humified and non-humified fractions

Table 5. Organic matter fractions and humification parameters in the initial mixture (IM) and in the final worm-worked (W) and non-worm-worked (NW) products. (TEC: total extractable carbon, NH: non-humified fraction, HA: humic acids, FA: fulvic acids, HI: humification index, DH: degree of humification, HR: humification rate)

		After 40 days	
	IM	W	NW
TEC, g kg^{-1}	33c	76a	65b
NH, g kg^{-1}	13c	31b	27b
HA, g kg^{-1}	16b	31a	30a
FA, g kg^{-1}	3.2c	13a	6.5b
TEC/TOC, %	7.8	31a	22b
HI	0.68a	0.70a	0.74a
DH, %	58a	58a	56a
HR, %	4.5c	18a	12b

All data are expressed on dry matter basis
Values in the same row followed by the same letter are not significantly different ($P<0.05$).

of the initial- and end-products remained constant with respect to total extractable carbon. On the contrary, the humification rate increased significantly, reaching maximum values in the worm-worked product. This increase implies that the entire mass rather than a small portion of organic matter in the initial mixture was simultaneously involved in the stabilization process (Sequi *et al.*, 1991; Ciavatta & Govi, 1993). In any case, although the humification process was more apparent in the presence of earthworms, the degree of humification and humification rate of the final products were slightly lower than those generally found for organic matter extracted from well-mature organic amendments (De Nobili *et al.*, 1989).

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