Bioresource Technology 128 (2013) 193–198

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Vermistabilization of paper mill wastewater sludge using *Eisenia fetida*

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HIGHLIGHTS

- ▶ Vermistabilization caused significant changes in chemical constituents of paper mill sludge, resulting in a stable end product.
- ▶ The end material was rich in microbial population (fungal, bacterial and actinomycetes).
- ▶ The end product would be suitable for land applications.

ARTICLE INFO

Article history:
Received 11 June 2012
Received in revised form 30 August 2012
Accepted 8 October 2012
Available online 16 October 2012

Keywords:
Industrial sludge
Vermicompost
Wastewater treatment
Earthworm
Soil nutrient mineralization

ABSTRACT

Vermistabilization of paper mill wastewater sludge (PMS) spiked with cow dung (CD) at ratios of 0%, 25%, 50%, 75%, and 100% was carried out employing the earthworm, *Eisenia fetida*. A total of five treatments were established and changes in chemical and microbial properties of mixtures were observed. Vermistabilization caused decreases in total organic carbon, C:N ratio and cellulose by 1.2–1.5, 4.6–14.6, and 2.3–9.7-fold, respectively, but increases in pH, electrical conductivity, ash content, $_{tot}N$, $_{avail}P$, $_{tot}P$, $_{exch}K$, Ca, Na, and $N{-}NO_3^-$ of 1.06–1.11, 1.2–1.6, 1.3–1.6, 3.8–11.5, 4.1–6.5, 5.7–10.3, 1.7–2.0, 1.16–1.24, 1.23–1.45, 4.2–13.4-folds, respectively. PMS with 25–50% of CD showed the maximum mineralization rate. The fungal, bacterial and actinomycetes population increased 2.5–3.71, 3.13–8.96, and 5.71–9.48-fold, respectively after vermistabilization. The high level of plant-available nutrients indicates the suitability of vermistabilized material for agronomic uses.

1. Introduction

The pulp and paper industry is considered one of the most polluting industries (Thompson et al., 2001). A variety of liquid and solid wastes are produced during different processes of paper manufacturing. In general, pulping and bleaching are the two main steps in production of paper in industry and a huge quantity of fresh water is utilized resulting a large quantity of wastewater, sludge and other solids. The solid wastes from pulp and paper industries are mainly treatment sludges, lime mud, lime slaker grits, boiler and furnace ash, scrubber sludges, and wood processing residuals. The paper production generates around 45% wastewater sludge (0.2–1.2 kg dry matter (DM)/kg of biological oxygen demand (BOD) removed), 25% ash, (Zambrano et al., 2003), 15% wood cuttings and waste, and 15% other solid waste. The sludge from wastewater treatment units (20–60% solid fractions, $pH \approx 7$) includes wood fibers, biosludge, calcium carbonate, clay and other inorganic materials (Nurmesniemi et al., 2007). Dry sludge amounts to approximately 4.3% of the final product, increasing to 20–40% for recycled paper mills (World Bank, 2007). The primary methods of disposal for this type of sludge have been land application and landfilling. The unsafe disposal of these solid wastes cause environmental problems because of high organic content, partitioning of chlorinated organic, pathogens, ash and trace amounts of heavy metals. The pulp and paper industry faces a growing solid waste disposal problem as environmental regulations become increasingly stringent and landfill space grows scarcer. The chemical analysis (high organic matter content, pH, buffer capacity, nitrogen and phosphorous level, and low concentrations of heavy metals and organic pollutants) have revealed that pulp mill sludge may be utilized as a soil amendment, improving soil fertility (Zhang et al., 2004) but stabilization involving decomposition of an organic waste to the extent that biological and chemical hazards are eliminated is required (Benito et al., 2003; Suthar, 2010; Gomez-Brandon et al., 2011).

Composting or vermicomposting have been promoted as a potential methodology for generating a product from wastes that can be used as a soil amendment (Sinha et al., 2010). Vermicomposting involves the biooxidation and stabilization of organic materials but, in contrast to composting, it depends on the joint action of earthworms and microorganisms and does not involve a thermophilic stage (Dominguez, 2004). Loehr et al. (1985) concluded that in a vermicomposting system, the earthworms maintain aerobic

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http://dx.doi.org/10.1016/j.biortech.2012.10.022

conditions, ingest solids, convert a portion of the organics to worm biomass and respiration products and expel partially stabilized matter as discrete particles (vermicompost). Vermicomposting and composting can be used in sequence to take advantage of the unique and valuable features of each (Dominguez and Edwards., 2011). Utilization of epigeic earthworms for the decomposition of waste solids from paper mill has already been reported (Butt, 1993; Elvira et al., 1998; Kaur et al., 2010), but information on composting/vermicomposting of primary sludge form wastewater treatment unit of paper mill is still not investigated. The aim of the present investigation was to stabilize primary sludge from a wastewater treatment unit of a paper mill using the earthworm species, *Eisenia fetida*. The dried sludge was mixed with cow dung at different ratios in order to optimize the waste mixture for better decomposition/mineralization. The changes in physico-chemical and microbial parameters of waste materials were measured. Earthworm growth and cocoon production patterns in different waste mixture were also monitored during vermicomposting process.

2. Methods

2.1. *Eisenia fetida*, paper mill waste water sludge, cow dung

E. fetida was procured from the vermiculture unit, Indian Veterinary Research Institute, Izatnagar, India and cultured in a circular plastic container containing of 30 L of cow dung mixed with leaf litter.

Wastewater sludge was collected from the wastewater treatment unit of a paper mill (Sri Bardri Kedar Papers Pvt. Ltd., Nazibabad, Utter Pradesh, India). The paper mill sludge containing 65–74% moisture was collected in 20-L plastic containers, dried in the shade on polythene bags to remove excess water while turning daily. Partially dried sludge cake solids were homogenized and shredded.

Fresh cow dung (CD) was obtained from a cowshed located in Mothrawala, Dehradun, India. The cow dung was partially dried in a shed and stored for further experimentations. The characteristics of the CD and paper mill wastewater sludge (PMS) are presented in Table 1.

2.2. Vermicomposting trials

Dried PMS and CD were mixed to obtain the following substrates: T_1—PMS (25%) + CD (75%), T_2—PMS (50%) + CD (50%), T_3—PMS (75%) + CD (25%), T_4—CD (100%), and T_5—PMS (100%). Two-hundred g (dry weight basis) of the mixtures were filled in triplicate into 1.5-L circular plastic containers and moistened with distilled water to maintain a moisture level of 65–70%. The substrates were kept for one week for initial thermal stabilization, initiation of microbial degradation and softening of waste mixtures. For vermicomposting, fifteen earthworms (4- to 5-wk old) weighing 496–516 mg each was released into each container. The moisture level in waste mixtures was maintained around 60–65% throughout the study period by weakly sprinkling of an adequate quantity of water. The containers were placed in a humid and shady place at an ambient temperature (27–28 °C). Samples were drawn at 0, 7, 14, 21, 28, 35, 42, 49, and 56 days from each experimental container, dried in an oven for 48 h at 60 °C, and stored in sterilized plastic airtight containers at ambient temperatures.

Table 1
Chemical characteristics (g kg^{-1}) of paper mill sludge (PMS) and cow dung (CD) used in experimentations (mean ± SD, $n = 3$).

Parameters	PMS	CD
pH	7.58 ± 0.03	8.12 ± 0.01
EC (μ S)	2.78 ± 0.03	3.37 ± 0.03
TOC (g kg^{-1})	616.30 ± 1.25	675.47 ± 1.14
TKN (g kg^{-1})	2.39 ± 0.01	11.78 ± 0.01
K (g kg^{-1})	3.17 ± 0.01	9.75 ± 0.03
AP (g kg^{-1})	31.40 ± 0.32	33.85 ± 0.32
N—NO$_3^-$ (mg kg^{-1})	36.11 ± 4.24	143.51 ± 2.45
Ca^{2+} (mg kg^{-1})	59.73 ± 0.17	64.20 ± 0.34
TP (g kg^{-1})	0.54 ± 0.03	0.73 ± 0.07
Na$^+$ (g kg^{-1})	4.13 ± 0.02	5.63 ± 0.03
C:N ratio	289.47 ± 0.50	57.32 ± 1.52

Anonymous: EC, electrical conductivity; TOC, total organic carbon; TKN, total kjeldahl nitrogen; AP, available phosphorous; and TP, total phosphorus.

Biomass, cocoon production rate, mortality, and fecundity rate of the earthworms were measured as described by Singh and Suthar (2012).

2.3. Chemical analysis

The pH was measured using a digital pH meter (Metrohm, Swiss made) in 1:10 (w/v) aqueous solution (deionized water). The EC of water-extracted samples was measured using a digital conductivity meter. Total organic carbon (TOC) was measured after igniting the sample in a Muffle furnace at 550 °C for 60 min (Nelson and Sommers, 1996). Total Kjeldahl nitrogen (TKN) was measured by the method of Jackson (1975). Nitrate N—NO$_3^-$ was measured spectrophotomatrically after extracting samples using 0.01 M CuSO$_4$ solution (Jackson, 1975). Available phosphorous (AP) and total phosphorus (TP) were measured using the method described by Olsen et al. (1954). Calcium (Ca) and exchangeable potassium (K) were determined after extracting the sample with ammonium acetate (Simard, 1993). Sodium (Na) was estimated by the method of Jackson (1975) using a flame photometer. The cellulose content was estimated as described by Carter and Gregorich (2008). The biodegradability coefficient (K_b) was calculated using the following equation (Diaz et al., 1996): OM = (100 – Ash content%); K_b = (OMi – OMf) 100/OMi (100 – OMf) where OMi and OMf are the organic matter at initial and at final of vermicomposting process, respectively.

2.4. Microbial analysis

Total fungal populations, actinomycetes and aerobic bacterial populations were analyzed as described by Aneja (2004). One gram of material from each type of waste mixture was transferred to autoclaved test tubes containing sterilized distilled water and mixed thoroughly using horizontal shaker for 30 min. The mixture was diluted serially and 1-mL aliquots were pour-plated in Nutrient Agar (agar, 20.0 g; peptone, 10.0 g; NaCl, 5.0 g per 1 L of distilled water, Rose Bengal Agar (dextrose, 10.0 g; peptone, 5.0 g; K_2HPO_4, 1.0 g; $MgSO_4.7H_2O$, 0.5 g; Rose Bengal, 0.5 g; agar, 15.0 g per L of distilled water), and Kenknight's medium (glucose, 1.0 g; $NaNO_2$, 0.1 g; K_2HPO_4, 0.1 g; $MgSO_4.7H_2O$, 0.1 g; KCl, 0.1 g; $(NH_4)_2SO_4$, 0.1 g; agar, 15.0 g per L of distilled water). Plates were incubated for 24, 72 h, and one week, respectively to count the CFUs (colony forming units) of bacteria, fungi, and actinomycetes, respectively.

2.5. Statistical analysis

One-way ANOVA was used to analyze the differences between treatments. A Tukey's *t*-test was also performed to identify the homogeneous type of the data sets. SPSS® statistical package (Window Version 13.0) was used for data analysis. All statements reported in this study are at the $p < 0.05$ levels.