Assessment of qualitative enrichment of organic paper mill wastes through vermicomposting: humification factor and time of maturity

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ABSTRACT

The process of bioconversion of solid organic wastes through vermicomposting justifies the environmental message for sustainability such as reduce, recycle and reuse of wastes. In the present study, wastes derived from two different types of paper mill sludge (primary and secondary), was used for their bioconversion through the vermicomposting process using an indigenous species of earthworm (*Perionyx excavatus*). The maturity and stability stage of vermicompost production was assessed using FT-IR, GC-MS and TG analyses. During vermicomposting, different biochemical functional groups present in the wastes have shown differential chemical alteration and turnover as revealed by FT-IR spectroscopy. This study has also confirmed the trend of biodegradation of complex substances like lignin, cellulose, proteins etc. and thereby demonstrates the extent of mineralization. TG spectral analysis had revealed a mass loss of 80% and 71% in vermicompost produced from primary and secondary sludge respectively. GC-MS studies have also shown the presence of several humic acids like octadecanoic acid, heptadecanoic acid etc. in the decomposing substances demonstrating as an indicator of the maturity of products. This was further confirmed by the decrease of humification index which focuses the combined action of both earthworms and microbes in the degradation of organic wastes. The present study has highlighted the role of an indigenous earthworm in converting specific industrial wastes especially by recording the point of maturation using humic acids an indicator of the quality of decomposing of wastes following several instrumental applications.

1. Introduction

With the advent of science and expanding urbanization, proper management of solid wastes has become a crucial and challenging problem in the context of environmental management. The improper eco-management leads to several health hazards and disturbs the sustainability of the ecosystem. Therefore, selection of proper economically feasible effective techniques specifically through biological means demands utmost priority. Using suitable species of earthworm, vermicomposting act as an effective biological process which converts complex organic wastes into a nutrient-enriched black gold or fertilizer known as vermicompost (Lalander et al., 2015; Yadav et al., 2011). During vermicomposting, an intricate relationship between earthworms and associated microbiota were found which help in bioconversion of toxic organic wastes into stable nutrient enriched organic products, popularly known as vermicompost (Bhat et al., 2013; Chakraborty, 2018; Ganguly and Chakraborty, 2018). Vermicompost has proved to be an efficient producer for plant growth regulators and nutrients in simple forms (Lim and Wu, 2016). Earthworms feed on organic wastes and increase the surface area which was exposed to microorganisms making decomposition easier (Albanell et al., 1988; Garg et al., 2006; Liu et al., 2005). Materials upon digestion show increased nutrient properties with proven benefit for agriculture purpose (Díaz et al., 2007). Therefore, the maturity of vermicompost has appeared to be an essential parameter because the immature and incomplete production may inhibit plant growth owing to its phytotoxic effects (Gomez Brandon et al., 2008). Determination of functional groups within compost will help confer some knowledge regarding its maturity and effectiveness. FT-IR spectroscopy technique is used to confirm the decomposition of several functional groups. This technique has been frequently applied in environmental analyses regarding degradation of organic wastes (Gupta and Garg, 2009). In FT-IR, presence or absence of several spectral bands along with the spectral shift of different functional groups imply the degree of mineralization (Lim and Wu, 2016). Therefore, this spectroscopic technique was used...
along with GC-MS to find out the entire change in chemical footprints during the process. Many previous studies had focused on the quality enrichment of vermicompost but the present study has dealt with the comprehensive idea of composting of paper mill sludge and tries to understand the turnover of different organic components using spectroscopic analysis like FT-IR and GC-MS. This study has

Table 1

| Properties       | Primary sludge (PS) | Secondary sludge (SS) | Cow dung | Vp Initial | Vp Final | Vs Initial | Vs Final |
|------------------|---------------------|-----------------------|----------|------------|----------|------------|----------|
| Colour           | Grey                | Grey                  | Brown    | Grey Light grey | Grey Light grey |
| pH               | 5.65 ± 0.06         | 6.23 ± 0.08           | 7.14 ± 0.02 | 6.39 ± 0.06a | 8.23 ± 0.06a | 6.89 ± 0.03b | 8.05 ± 0.05c |
| EC (ms/cm)       | 2.08 ± 0.91         | 2.13 ± 1.25           | 3.95 ± 0.45 | 2.95 ± 0.56b | 1.75 ± 0.23c | 2.65 ± 0.45a | 1.45 ± 0.56b |
| TOC (gm/kg)      | 756 ± 0.56          | 410.32 ± 0.89         | 260 ± 1.23 | 735 ± 1.25c | 280.69 ± 1.35d | 390.23 ± 0.56c | 160.36 ± 0.25d |
| TKN (gm/kg)      | 6.23 ± 0.30         | 5.86 ± 0.20           | 17.86 ± 0.26 | 5.56 ± 0.26c | 23.28 ± 0.68d | 4.26 ± 0.20d | 24.36 ± 0.65e |
| C/N ratio        | 121.35 ± 1.87       | 70.02 ± 4.45          | 14.56 ± 4.73 | 132.19 ± 4.80d | 12.06 ± 2.0e | 91.60 ± 2.80d | 6.58 ± 0.38e |

Values are taken as mean ± SD, n = 3. Vp and Vs represent vermicompost of primary sludge and secondary sludge respectively. Different letters indicate significant differences at p = 0.05.

Fig. 1. FT-IR spectra of (a) 0th day (b) 30th day and (c) 60th day of vermicomposting of primary paper mill sludge. The number represent wavenumber of different functional groups during the course of vermicomposting.
also put special emphasis on the rate of humification during the process of vermicomposting using two different paper mill sludge such as primary and secondary ones, over a period for 60 days using an indigenous earthworm species *Perionyx excavatus*.

### 2. Materials and methods

#### 2.1. Experimental designs and physiochemical analysis

Primary sludge (PS) and secondary sludge (SS) samples were collected from UNIGLOBAL paper mill (Jhargram, India; 22° 27’ 0” N,
Two kilograms from each sample were properly sundried and mixed with cow dung and straw in a ratio of 5:4:1. Owing to the toxicity of sludge, samples were supplemented with cow dung which act as a bulking agent in ceramic tubs (24 inch × 18 inch ×18inch) along with thirty potential breeders of Perionyx excavatus for 60 days in three replicates (Ganguly et al., 2018). During the process of composting, the temperature was kept at 25 °C, pH (7.4-7.6) and moisture content of 60% through spreading of water at regular intervals (Ganguly and Chakraborty, 2018; Chanda et al., 2010).

Physiochemical analyses of both sludges were performed for initial and final stages respectively. Chemical analyses of two nutrients (Carbon/Nitrogen ratio) were considered as Total Organic Carbon (TOC) and Total Kjeldahl Nitrogen (TKN) using Walkley-Black and Kjeldahl methods respectively (Ganguly and Chakraborty, 2018). Electrical conductivity (EC) and pH of samples were measured by dissolving the samples in double distilled water in the ratio of 1:10 (w/v) using multitasking water analyzer (Cyber Scan series 600).

### 2.2. Fourier transform-infrared (FT-IR) spectrum analysis

FT-IR analysis was done to identify the turnover of different functional groups in the vermicompost of primary and secondary sludge at a definite interval within a frequency range of 4,000 - 400 cm⁻¹ on a Perkin-Elmer FT-IR spectrometer. 1 mg of the sample was finely mixed with the spectroscopic grade of 100 mg KBr and was pressed into a pellet.

![Fig. 4. GC-MS spectrum of (a) and (b) vermicompost of primary sludge at 0th and 60th day respectively.](image-url)
of 1mm thickness and 10 mm in diameter at a pressure of 1 Mpa (megapascal). After baseline correction with respect to control spectral bands were taken and studied to understand the chemical composition of the sample. The experimental procedure was followed according to Ravindran et al. (2013).

### 3.2. FT-IR analysis

Mineralization of organic matter and degradation of complex aromatics (lignin, polyphenols) by earthworms into simpler compounds (carbohydrates, lipids) can be analyzed by FT-IR spectroscopy. FT-IR

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Table 3

List of compounds identified from GC-MS spectra at 60th day of vermicompost prepared from primary paper mill sludge.

| Peak | R.Time | Area % | Compound Name |
|------|--------|-------|---------------|
| 1    | 15.618 | 10.47 | 1,2-benzenedicarboxylic acid, diethyl ester |
| 2    | 15.717 | 0.81  | Tris(tert-butyldimethylsilyloxy)-1,3,5-trimethyl-1,3,5-bis[trimethylsilyl]oxo]oxalanone |
| 3    | 19.211 | 15.82 | Heptadecanoic acid, 16-methyl, methyl ester |
| 4    | 19.558 | 0.81  | Trisiloxane, 1,1,5,5,9,9,9,9,9,9-tetramethyl- |
| 5    | 19.758 | 4.06  | 1,2-benzenedicarboxylic acid, dibutyl ester |
| 6    | 19.892 | 1.21  | Hydroxyurea, N,N,N-Trimethyl urea |
| 7    | 19.975 | 0.78  | Benzo[fluorine]-2,3-bis(pyridine-1(2h)-carboxylic acid, 3-2,3-dimethyl-4-methyl, methyl ester |
| 8    | 20.894 | 2.30  | Isophthalic acid, pentadecyl-4-ethyl ester |
| 9    | 21.042 | 3.85  | Carnosine |
| 10   | 21.083 | 2.17  | 1,1,1,3,5,5,7,7,7-Octanenonitrile-3(trimethylsilyloxy)-tetrasiloxane |
| 11   | 21.271 | 11.83 | Octadecanoic acid, methyl ester |
| 12   | 21.475 | 2.82  | N-methoxy-N-benzoyl-1,1-dimethyl-2-carboxymethoxyethylamine |
| 13   | 22.152 | 0.88  | Imidazol-2-carboxylic acid, 2-amino- |
| 14   | 22.493 | 2.40  | Propanedioic acid, hexyl-, diethyl ester |
| 15   | 22.620 | 0.24  | 1,7-Di(2,2-dimethylphenyl)-2,2,4,4,6,6-hexamethyl-1,3,5,7-tetraoxo-2,4,6-trisiloxane |
| 16   | 22.683 | 0.77  | Cyclohexanecarboxamide, N-furfuryl- |
| 17   | 22.808 | 0.77  | Benzenacetic acid, alpha,3,4-tris(trimethylsilyloxy)-, trimethyliyl N-

*R.Time is Retention Time.

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Table 4

List of compounds identified from GC-MS spectra at 0th day of secondary paper mill sludge.

| Peak | R.Time | Area % | Compound Name |
|------|--------|-------|---------------|
| 1    | 8.200  | 9.42  | Undecane |
| 2    | 15.602 | 14.43 | 1,2-Benzenedicarboxylic acid, diethyl ester |
| 3    | 16.474 | 2.63  | Pentadecanal |
| 4    | 19.761 | 5.62  | 2-(3-enolyl)benzoic acid, benzonic acid |
| 5    | 19.883 | 2.73  | 4,5,7-Trihydroxy-2-octenoic acid |
| 6    | 20.892 | 4.06  | 9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-yl)methyl ester, cis- |
| 7    | 20.967 | 3.04  | Tartaric acid |
| 8    | 21.041 | 4.14  | Ethyl Oleate |
| 9    | 21.758 | 0.93  | 2-Hexanone, 3-cyclohexylidene-4-ethyl- |
| 10   | 21.958 | 1.55  | 1,3-Carboxyphosphonate, 2-butyl-5,5-bis(ethylcarboxybiyl) |
| 11   | 22.502 | 2.17  | Hexadecane, 1,16-dichloro- |
| 12   | 22.577 | 2.85  | 1,2,2,3,3,6,6,6-Octafluorohexane, 3,3,3-trifluoro-2-ethynyloxy- |
| 13   | 22.877 | 2.58  | 1,2-benzenedicarboxylic acid, diocetyl ester |
| 14   | 29.418 | 1.11  | Silane, (4-ethylphenyl)trimethylsilane |
| 15   | 29.608 | 1.09  | Tri(tert-butylmethylsilyloxy)arsane |

*R.Time is Retention Time.

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2.3. UV – vis spectroscopy analysis

The extent of humification during vermicomposting of primary and secondary sludges was estimated using UV-Vis spectroscopy (SHIMADZU UV – 1601). 1 gm each of vermicompost was taken and extracted using 50 ml of 1M NaOH through vigorous shaking for 3 h and kept overnight undisturbed. The solution was then centrifuged at 3,000 rpm for 40 min and absorbance was recorded at 470 nm (A472), and 660 nm (A660). Absorbance at 472 nm reflects the beginning of humification whereas A660 demonstrate highly humified material with aromatic compounds. Therefore, the ratio of A472 and A660 represents the extent of humification and known as humification index (Qh) (Giegeuzska et al., 1998).

2.4. Gas chromatography and mass spectrophotometry (GC-MS) analysis

5 mg of each vermicompost samples were mixed with 100 ml of methanol-water (9:1) (v/v) and were left overnight. Samples were then filtered and evaporated using a vacuum evaporator and dissolved in methanol (Hussain et al., 2016). 1 µl of samples were injected into the gas chromatograph of GC-MSQP2020. Peaks obtained from the samples were then compared using Wiley online library.

2.5. Thermogravimetry (TG) analysis

Without any treatment, 50 mg of different vermicompost samples were grounded and sieved to less than 2.0 mm size. TG analysis was done to understand the stability of sample using analyzer model DTG-60. The analysis was carried out within a temperature range of 30–1000 °C in a nitrogen atmosphere with a heating rate of 10 °C/min at 101 kPa manometric pressure (El Ouaqoudi et al., 2015).

2.6. Statistical analysis

Tukey test (One-way ANOVA) was performed using SPSS version 25. These tests were performed to find out any significant relation between the change in different physiochemical parameters.

3. Results

3.1. Physiochemical analysis

A significant change in physiochemical parameters was observed during the process of vermicomposting (Table 1). Initially pH was found to be acidic in both primary sludge (pH = 6.39) and secondary sludge (pH = 6.89) and subsequently attained a higher value of pH = 8.23 (PS) and pH = 8.05 (SS) at 60th day of vermicomposting. The significant increase in pH reflects a huge breakdown of fatty acids and precipitation of mineral salts along with cation complexed humic acids (Tognetti et al., 2007; Brady and Weil, 2002). Electrical conductivity (EC) for initial feed mixture were found to be high for both PS (29.5) and SS (26.5) which was found to decrease significantly as 1.75 (PS) and 1.45 (SS). The declining trend of electrical conductivity reflects the stability of vermicompost (Singh et al., 2010). Initially, the C/N ratio for primary and secondary sludge was found to be 132.19 and 91.60 respectively, which finally goes to 12.06 and 6.58 for both types of sludges respectively. The declining trend of electrical conductivity reflects the stability and extent of decomposition by the microbes (Ganguly and Chakraborty, 2018; Yadav et al., 2013).

3.2. FT-IR analysis

Mineralization of organic matter and degradation of complex aromatics (lignin, polyphenols) by earthworms into simpler compounds (carbohydrates, lipids) can be analyzed by FT-IR spectroscopy. FT-IR
spectroscopy technique indicates compost maturity or stability and has appeared to be a promising technique for the identification of functional groups in the decomposing wastes in the process of vermicomposting. Several spectral bands represent the absence or presence of certain functional groups which in turn predict the processes of degradation or stabilization and therefore act as a promising tool to diagnose the maturity and stability of the sample (Fig. 1 and Fig. 2).

FT-IR spectra during different times (0th, 30th and 60th days) of composting for both sludges have displayed an intense wide band in between 3200 - 3500 cm$^{-1}$ which indicates an $\text{-OH}$ stretching of acid, phenols and alcohols group (Pandey and Pitman, 2003; Amir et al., 2010; Hussain et al., 2016). The intensity of the band decreases with the progress of vermicomposting for both types of paper mill sludge. Spectral bands between 3000 - 2840 cm$^{-1}$ represent $\text{C–H}$ stretching of aliphatic compounds mainly represented by alkenes (Deka et al., 2011). A sharp decline of this to a considerable extent reflects evidence of intense biodegradation coupled with compost maturity (Fig. 1c and Fig. 2c). The decrease in the intensity of the band at 2531 cm$^{-1}$ of both the composts represent a breakdown of the complex $\text{–SH}$ groups of paper mill wastes (Sudarshan et al., 2016).

Furthermore, the efficiency of the earthworm species, Perionyx excavatus was highlighted with the increase in band intensity at 2359 cm$^{-1}$ representing the degradation of inorganic silanes (Chuprov et al., 2006). Silanes were used as coupling agents of paper fibres in an industrial operation of paper pulp industries. Increase in spectral band

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Fig. 5. GC-MS spectrum of (a) and (b) vermicompost of secondary sludge at 0th and 60th day respectively.

| Peak | R.Time | Area % | Compound Name |
|------|--------|-------|----------------|
| 1    | 20.864 | 5.63  | Phthalic acid, heptyl 2-methoxyethyl ester |
| 2    | 20.975 | 6.84  | (2-Acetoxyethyl) trimethylammonium |
| 3    | 21.108 | 7.58  | Benzoic acid, 3-methyl-2-trimethylsilyloxy-, trimethylsilyl ester |
| 4    | 21.206 | 8.16  | (±)-Carpesiolin |
| 5    | 21.335 | 8.00  | Benzoex |
| 6    | 21.510 | 8.70  | 1,2-Benzenediol, 3,5-bis(1,1-dimethylethyl) - |
| 7    | 21.717 | 8.44  | Benzoic acid, 2,3-bis[(trimethylsilyl)oxy]-, trimethylsilyl ester |
| 8    | 21.825 | 7.43  | Silane,[[4(1,2bis[[trimethylsilyl]oxy]ethyl]1,2-phenylene]bis[ox]]bis[trimethyl- Methyprylene |
| 9    | 22.033 | 6.45  | Decane, 1,9-bis[[trimethylsilyl]oxy]- |
| 10   | 22.217 | 6.89  | 1,4-Benzodioxin, 6-buty1-2,3-dihydro-5-(2- methylaminomethoxy)- |
| 11   | 22.442 | 5.81  | Silane,[[4(1,2bis[[trimethylsilyl]oxy]ethyl]1,2-phenylene]bis[ox]]bis[trimethyl- Methyprylene |
| 12   | 22.584 | 5.39  | Cyclohexane, eicosyl |
| 13   | 22.872 | 4.56  | 1,1,3,5,5,7,7,9,11,11,13,13-Tetradecamethyl- heptasiloxane |
| 14   | 23.152 | 2.85  | Ethanol, 1-(methylene cycloproplyl)-1-(methylene-1-trimethylsilyl cycloproply)- |
| 15   | 28.793 | 7.25  | 1,2-Benzenedicarboxylic acid |

*R.Time is Retention Time.*
intensity at 1740–1870 cm\(^{-1}\) during the process of vermicomposting indicate C=O of different anhydrides and esters (Paria et al., 2018). Besides that, amide band at 1635 cm\(^{-1}\) of varying intensities tend to focus biodegradation of complex N sources from both the composts (Barone and Arikan, 2007). Band peaks at 1420 - 1440 cm\(^{-1}\) and 1000 - 1100 cm\(^{-1}\) are thought to correspond lignin vibrations and the C=O stretch of polysaccharides respectively (Fig. 1 and Fig. 2). Varying intensities of different bands during vermicomposting represent the degradation of lignin and complex carbohydrates like cellulose, hemicellulose etc. (Ravindran et al., 2013). The increase in the aromatic C to aliphatic C as reflected from FT-IR curves is considered as an indicator of an increasing degree of organic matter humification in the natural condition of biodegradation which is supposed to be associated with the stability and maturity of compost and their pathways of transformation to highly humified substrate (Senesi and Brunetti, 1996; Huag et al., 2006).

In such context, upon the comparison of spectral bands during vermicomposting of the sludge, it can be inferred that change in respective bands was much prominent and conspicuous for secondary sludge in comparison to primary ones. It can also be ascertained that treatment of primary sludge during waste management make secondary sludge less toxic and best feed for the earthworm species, *Perionyx excavatus*.

### 3.3. UV-vis spectroscopy analysis

Spectroscopic analysis was used to determine the maturity and humification of the sample during the final phase of vermicomposting. Our recent findings have established the role of microbes in liberation a couple of enzymes during composting which may facilitate the formation of humic acids (Ganguly and Chakraborty, 2018). Humification index /A\(_{472}/A\_460\) decreases upon composting as compared with the initial. Lowering of ratio was assumed to be due to high condensation of aromatics and low occurrence of aliphatics (Fig. 3). This ratio shows an inverse proportional to extent of aromaticity, size of particle etc. Therefore, it clearly indicates a high degree of degradation, oxidation, and decomposition as well as stabilization of final compost through the enrichment of humus (Lim and Wu, 2015).

### 3.4. GC-MS analysis

The GC-MS study reveals the chemical footprint of the samples in terms of vermicomposting. For the compost of primary sludge, 18 peaks were recorded at the beginning with maximum peak area benzoldi-carbonsaeure (Table 2 and Fig. 4a). After vermicomposting for 60 days the number of peaks rose to 25 with maximum peak areas for Heptadecanoic acid, Octadecanoic acid, and Benzene dicarboxylic acid (Table 3 and Fig. 4b). This evidently shows that vermicomposting results in the enrichment of humic acids like substances. For the vermicomposting of secondary sludge, 20 peaks were recorded at the beginning with a maximum peak area of Benzene dicarboxylic acid (Table 4 and Fig. 5a). After 60 days peak number get decreased to 15 peaks with the maximum peak area for benzene-di-ol (Table 5 and Fig. 5b). The numbers of peaks indicate the extent of composting which implies the degradation of large molecular mass. The appearance of humic acids in the compost samples indicates the extent of maturity of the sample as explained previously by the others. Enrichment of humic acids and plant growth hormones in final vermicompost were proved previously by several authors (Hussain et al., 2016).

#### 3.5. TG analysis

TG curves determine the stability of vermicompost through the differential loss of mass under different temperature during vermicomposting. Spectra demonstrate a mass reduction of almost 80% for primary sludge and 71% for secondary sludge vermicompost respectively. A dehydration reaction between temperature 60–100 °C and 200–700 °C for decomposition in all treatments were found (Korosec and Lavric, 2009). Reduction of mass loss under different temperature represents the stability of vermicompost. Vermicompost material at the final stage shows a reduction in the mass loss as compared with the initial. Breakdown of long-chain aliphatics, saccharides and proteins were noticed within the temperature range of above 400 °C, 250–500 °C and 330 °C respectively (Fig. 6.). Previously many authors demonstrate an increased weight loss of humic acids due to microbe-earthworm interactions (Ravindran et al., 2013, Fernandez-Gomez et al., 2015; Lim and Wu, 2016). These findings attribute to the enhanced mineralization by earthworms over sludge material.

### 4. Conclusion

The present study tries to convey the importance of indigenous species of earthworm from India, *Perionyx excavatus* for the purpose of sustainable organic solid waste management. Under proper environmental conditions, the study reflects a sharp decline of C/N ratio upon maturation of vermicompost. The detailed instrumental applications like FT-IR and GC-MS have pin-pointedly justified the efficacy of indigenous species in the turnover of functional groups as well as an amendment as a source of the enrichment of humic acid-like substances for vermicomposting over other exotic species. Lowering of humification index...
during the period of composting reflects the rise of aliphatic C from aromatic C. TG curves demonstrate the extent of maturity and mineralization during vermicomposting. A better efficacy in the bioconversion of organic waste (primary sludge) was noticed as compared with secondary sludge. Therefore, the present study can be claimed to be a pioneering attempt in an interpretation of the role of indigenous species for its effective role in the bioconversion of two different paper mill sludge.

Declarations

Author contribution statement

Ram Kumar Ganguly: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Susanta Kumar Chakraborty: Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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