Journal of Hazardous Materials 205–206 (2012) 24–31

Contents lists available at SciVerse ScienceDirect

Journal of Hazardous Materials

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

Changes in the chemical characteristics of water-extracted organic matter from vermicomposting of sewage sludge and cow dung

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ARTICLE INFO

Article history:
Received 22 September 2011
Received in revised form
14 November 2011
Accepted 21 November 2011
Available online 28 November 2011

Keywords:
Vermicomposting
Water-extractable organic matter (WEOM)
Gel filtration chromatography
^{1}H nuclear magnetic resonance
Fluorescence spectra

ABSTRACT

The chemical changes of water-extractable organic matter (WEOM) from five different substrates of sewage sludge enriched with different proportions of cow dung after vermicomposting with *Eisenia fetida* were investigated using various analytical approaches. Results showed that dissolved organic carbon, chemical oxygen demand, and C/N ratio of the substrates decreased significantly after vermicomposting process. The aromaticity of WEOM from the substrates enhanced considerably, and the amount of volatile fatty acids declined markedly, especially for the cow dung substrate. Gel filtration chromatography analysis showed that the molecular weight fraction between 10^3 and 10^6 Da became the main part of WEOM in the final product. ^{1}H nuclear magnetic resonance spectra revealed that the proportion of H moieties in the area of 0.00–3.00 ppm decreased, while increasing at 3.00–4.25 ppm after vermicomposting. Fluorescence spectra indicated that vermicomposting caused the degradation of protein-like groups, and the formation of fulvic and humic acid-like compounds in the WEOM of the substrates. Overall results indicate clearly that vermicomposting promoted the degradation and transformation of liable WEOM into biological stable substances in sewage sludge and cow dung alone, as well as in mixtures of both materials, and testing the WEOM might be an effective way to evaluate the biological maturity and chemical stability of vermicompost.

1. Introduction

Vermicomposting, involving the joint action of earthworms and microorganisms [1–3], considerably improve the decomposition and stabilization of sewage sludge. The end products in the vermicomposting process were found to have higher N and P contents [4,5] and lower human pathogen [6,7], yielding an organic amendment or a soil conditioner. Vermicompost, as a soil organic amendment, could improve the physical, chemical, and microbial properties of the soil, and stimulates plant growth [8–14].

Previous studies on vermicomposting focused on the stabilization of sewage sludge, evaluation of vermicompost as a soil organic amendment or fertilizer, and earthworm population dynamics [15–17]. To date, the influence of vermicomposting on chemical features of the water-extracted organic matter (WEOM) in sewage sludge, and added enrichments such as cow dung, has not been fully studied.

During vermicomposting, microbes are responsible for biochemical degradation of organic matter, while earthworms are the critical drivers of the process, conditioning the substrate and altering the microbiological activity [18]. Since the biochemical transformations of organic matter are a result of microorganisms whose metabolism occurs in the water-soluble phase [19], WEOM represents the most active fraction of sewage sludge, and could directly reflect the biochemical alteration of organic matter. The concentration and chemical changes of WEOM have been found to correlate closely to the stability and maturity of compost during composting [19–22]. Thus, investigating the chemical changes of WEOM during vermicomposting process is highly desirable to better understand the stabilization of sewage sludge and the role played by the WEOM in that process.

The changes in the chemical characteristics of WEOM during composting process have been reported by a number of researchers [19,21–24]. Many analyses, including dissolved organic carbon (DOC), specific ultra-violet absorbance (SUVA), gel permeation chromatography (GPC) and fluorescence excitation–emission matrix spectroscopy (EEM), are used to investigate the properties of WEOM [22]. DOC can serve as a general descriptor of WEOM [21], while SUVA, GPC, ^{1}H NMR and EEM techniques could supply more information on WEOM transformation, e.g., the aromaticity, molecular weight and humification degree of derived compounds [22,24–28].

Volatile fatty acids (VFAs) were regarded as the major malodorous compounds in livestock manure [29] and sludge, and

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doi:10.1016/j.jhazmat.2011.11.070

could provide useful information about the volatile organic matter. However, information about VFAs changes in WEOM during vermicomposting of sewage sludge is still limited.

The aims of this work were to study the changes in the chemical characteristics of WEOM fractions after the vermicomposting of sewage sludge, and added enrichment such as cow dung, by various analytical approaches.

2. Materials and methods

2.1. Substrates and vermicomposting process

Fresh sewage sludge (FSS) was procured from of the dumping site of a 60 000 $m^3\ d^{-1}$ domestic waste water treatment plant (WWTP) (Quyang WWTP, Shanghai, China). The water content of the FSS approximated 75–85%. Fresh cow dung (FCD) was obtained from a cow farm in a suburb of the Pudong district, Shanghai, China. The FSS and FCD were dried in direct sunlight for two weeks, with periodic turning. Young non-clitellated earthworms *Eisenia fetida* were randomly picked from several stock cultures maintained in the laboratory with cow dung as culturing substrate.

Six circular plastic containers (15 cm diameter × 14 cm depth) were filled with 100 g feed mixture (dry weight) containing different proportion of sewage sludge and cow dung (V1, 100% + 0%; V2, 70% + 30%; V3, 50% + 50%; V4, 30% + 70%; V5, 0% + 100%). 10 g straw (dry weight) was added to the feed mixture as bulking material. These mixtures were manually turned every 24 h for 14 days in order to eliminate volatile toxic substances. After 14 days, 15 non-clitellated earthworms, weighing 200–250 mg live weight, were introduced in to five distinct groups (from V1 to V5), three replicates each. Mixture moisture content was maintained at 70–90% by periodic sprinkling of distilled water. All the containers were kept in the dark at room temperature (25 ± 1 °C). All containers were vermicomposted for 60 days followed by separation of earthworms, cocoons and straw. Vermicomposts were collected from each container for further analyses.

2.2. Extraction of water-extracted organic matter (WEOM)

WEOM was extracted from the initial substrates and vermicomposts with deionized water (solid to water ratio of 1:20) for 24 h on a horizontal shaker at room temperature. The suspensions were then centrifuged at $10^4 \times g$ for 10 min and filtered through a 0.45-μm Whatman® membrane filter [19].

2.3. WEOM analyses

2.3.1. Organic matter contents analysis

Dissolved organic carbon (DOC) and dissolved total nitrogen (DTN) of the filtrates were measured by a TOC-VCPN analyzer (Shimadzu, Japan). Dissolved chemical oxygen demand (DCOD) was measured using a NOVA60 COD meter (Merck, Germany). UV absorption at 254 nm of bulk WEOM samples was measured using a UV 765 spectrophotometer (Shanghai Precision & Scientific Instrument Co., Ltd., Shanghai, China). Before measurement, all solutions were diluted to organic C concentrations <10 mg L^{-1} [24]. The measured absorbance was normalized to the concentration of dissolved organic C giving the specific UV absorption ($SUVA_{254}$) [30].

2.3.2. Volatile fatty acids (VFAs) analysis

VFAs of the WEOM from the substrates were measured according to the method [31]. In brief, the filtrate was collected in a 1.5 ml gas chromatography vial and 3% H_3PO_4 was added to adjust the pH to approximately 3.0. A HP5890 Gas Chromatograph (GC, Thermo Finnigan, USA) with flame ionization detector and equipped with a 30 m × 0.32 mm × 0.25 mm CPWAX52CB column was used to analyze the composition of VFAs. N_2 was the carrier gas at a flux of 50 ml min^{-1}. The injection port and the detector were maintained at 200 and 220 °C, respectively. The oven of the GC was programmed to begin at 110 °C and after 2 min, increase at a rate of 10 °C min^{-1} to 200 °C, and to hold at 200 °C for 2 min. The sample injection volume was 1.0 μl. Three replicates were measured for each sample.

2.3.3. Gel filtration chromatography (GFC) analysis

The molecular weight of the WEOM was measured by a GFC analyzer. The GFC system consisted of an Lc-10ADVP type gel column (Shimadzu, Japan). The TSKgel G4000PWXL column (Tosoh, Japan) was used, which was suitable for analyzing water-soluble polymers. Polyethylene glycols (PEGs) with molecular weight (M_W) of 1 169 000, 771 000, 128 000, 11 840, 4020, 620, and 194 Da (Merck Corporation, Germany) were used as calibration standards [22].

2.3.4. ^1H nuclear magnetic resonance (^1H NMR) spectra analysis

The WEOM was analyzed for ^1H NMR spectra using a 500 MHz spectrometer (Bruder GmbH, Karlsruhe, Germany) at room temperature. 2 ml of the filtrate was taken and freeze-dried. The freeze-dried fractions were dissolved in 0.5 ml D_2O. The ^1H NMR spectra were measured at a spectrometer frequency of 500.13 MHz, 2 s acquisition time. Chemical shifts were depicted relative to the resonance of tetramethylsilane. MestReNova 8.0 software was used to analyze the ^1H NMR spectral data.

2.3.5. Fluorescence spectra analysis

The WEOM was diluted with 0.1 mol L^{-1} phosphate buffer (pH 7.0) and the final DOC was made up to approximately 10 mg L^{-1} [22], and analyzed for fluorescence spectra, using F-4600 fluorescence spectrophotometers (Hitachi, Japan). Emission and excitation slits were set at a 10-nm band width, and a scan speed of 500 nm min^{-1} was selected for both monochromators. Conventional emission spectra were recorded over the range 380–550 nm at a constant excitation wavelength of 360 nm. Excitation spectra were obtained over a scan range of 300–500 nm by measuring the emission radiation at a fixed wavelength of 520 nm [2]. Emission and excitation slits were set at a 5-nm band width, and a scan speed of 12 000 nm min^{-1} was selected for both monochromators. The excitation–emission matrix (EEM) spectra were recorded by scanning the emission wavelength over the 250–600 nm range, while the excitation wavelength was increased sequentially from 200 to 500 nm. The voltage of photomultiplier tube (PMT) was set as 750 mV for low-level light detection. Surfer 8.0 software was used to analyze the data.

2.4. Statistical analysis

One-way ANOVA was used to analyze the differences among treatments.

3. Results and discussion

3.1. Organic matter contents

The organic matter content of WEOM from the initial substrates and vermicomposts is displayed in Table 1. Compared to observations in the FCD, FSS had higher DOC, DCOD and DTN contents, lower $SUVA_{254}$ value and C/N ratio.

After vermicomposting, DOC and DCOD contents and the C/N ratio decreased in all mixtures, ranging from 2.51 to 3.93 mg g^{-1}, 4.98 to 9.70 mg g^{-1} and 0.52 to 0.82 respectively, possibly attributed to the degradation of labile organic matter, particularly carbohydrates, amino sugars and low-molecular weight organic

Table 1
Content of water-extracted organic matter from fresh sewage sludge and cow dung (FSS and FCD), and the vermicomposts of different-proportion mixtures of sewage sludge and cow dung (100% + 0%, 70% + 30%, 50% + 50%, 30% + 70%, and 0% + 100%; V1, V2, V3, V4 and V5, respectively).

WEOM	DOC ($mg\,g^{-1}$)	DCOD ($mg\,g^{-1}$)	$SUVA_{254}$ ($L\,mg^{-1}\,m^{-1}$)	DTN ($mg\,g^{-1}$)	C/N
Initial substrates					
FSS	15.86 ± 0.13	84.84 ± 0.68	0.43 ± 0.01	7.34 ± 0.12	2.16
FCD	4.25 ± 0.04	9.78 ± 0.22	1.66 ± 0.02	0.81 ± 0.03	5.25
Vermicomposting					
V1	3.93 ± 0.06	9.70 ± 0.20	0.89 ± 0.03	7.92 ± 0.16	0.71
V2	3.75 ± 0.04	6.9 ± 0.10	0.93 ± 0.02	7.27 ± 0.021	0.52
V3	3.11 ± 0.02	4.98 ± 0.12	1.20 ± 0.03	5.77 ± 0.20	0.54
V4	2.63 ± 0.06	5.52 ± 0.06	1.69 ± 0.04	3.73 ± 0.10	0.71
V5	2.51 ± 0.01	4.98 ± 0.04	1.90 ± 0.05	3.07 ± 0.17	0.82

acids [22,24], and the repolymerization and condensation of soluble organic matter that lead to the formation of complex organic substrates with low solubility in water which tend to flocculate out the solution [32,33]. The DOC contents in all final vermicomposts were less than $4\,mg\,g^{-1}$, and tended to decrease from V1 to V5, which has previously been suggested as an indicator of maturity [21,22]. According to the previous results about mature composts [21,34], we suggest a threshold DOC value of $4\,mg\,g^{-1}$ for mature vermicomposts.

In the experiments, $SUVA_{254}$ and DTN content increased in the substrates after vermicomposting; the former ranged from 0.89 to $1.90\,L\,mg^{-1}\,m^{-1}$ while the latter changed from 3.07 to $7.92\,mg\,g^{-1}$. In the past, $SUVA_{254}$ has served as an indicator of the aromatic character of dissolved organic matter [26,30]. So, the results indicate that vermicomposting led to increase aromaticity in the WEOM and stabilization of sewage sludge, especially in V5.

Vermicomposting caused an increase in DTN contents and a decrease in the C/N ratio of the WEOM from the substrates, possibly resulted from strong degradation of organic carbon compounds [7,16]. Losses in organic carbon might be responsible for relative increase in nitrogen [35]. C/N ratio corresponds to the maturity degree of the studied organic material (compost, soil, sewage sludge) [36], and thus a decrease in C/N ratio implied an increase in maturity degree of the vermicomposts.

3.2. Volatile fatty acids (VFAs)

The contents of VFAs in WEOM from the initial substrates and vermicomposts are shown in Table 2. After the 10 d preparation period, the FSS were found to have five kinds of VFAs, including acetic acid, propionic acid, *n*-butyric acid, iso-butyric acid and *n*-valeric acid, while the FCD contained three components (acetic acid, propionic acid and *n*-butyric acid), whose contents were lower than that noted in FSS. Since the VFAs came from the fermentation of soluble organic matter in the digestion process [37], high content of VFAs in FSS indicated high amount of soluble organic matter, corresponding to DOC and DCOD results.

After vermicomposting, the VFA contents of the WEOM from all substrates decreased considerably in all substrates, and only one of VFA (acetic acid) was found in V3, V4 and V5, implying the volatile organic matter of the vermicomposts was substantially lost with the odor of the final products largely reduced, especially in V5. Som et al. [38] showed that volatile organic matter, such as low molecular weight acids (C_4 and C_5), were detected at the early beginning of the composting process, but these composts at the maturation phase were absent. Thus, these results indicated the maturity of the final vermicompost, and confirmed and complemented the previous findings about organic matter contents of WEOM.

3.3. Molecular weight distribution

Fig. 1 reveals the change in molecular weight distribution of the WEOM from the initial substrates and vermicomposts. After the vermicomposting process, the chromatograms of WEOM from the substrates indicated significant changes. In the initial substrates (FSS and FCD), there were 5 peaks in the chromatograms, while only 2–3 peaks were found in the chromatograms of vermicomposts. Vermicomposting likely caused the labile organic matter degradation most rapidly, and coupled with likely hydrolysis to smaller products, peaks of high-molecular weight fractions disappeared in the finial product.

All the chromatograms could be divided into three parts, namely the fractions with molecular weights below 10^3 Da, between 10^3 and 10^6 Da, and with molecular weights above 10^6 Da. Table 3 outlined the percentage distribution of these fractions in the initial substrates and vermicomposts.

After the vermicomposting process, the proportion of the fraction with molecular weights between 10^3 and 10^6 Da in all substrates increased considerably, and the percentage of the fraction with molecular weight above 10^6 Da decreased, suggesting the degradation/hydrolysis of this fraction of WEOM and an increasingly homogeneous mixture of compounds respect to molecular weight [22]. The increase might be attributed to the formation of

Table 2
Volatile fatty acid (VFA) content of water-extracted organic matter from fresh sewage sludge and cow dung (FSS and FCD), and the vermicomposts of different-proportion mixtures of sewage sludge and cow dung (100% + 0%, 70% + 30%, 50% + 50%, 30% + 70%, and 0% + 100%; V1, V2, V3, V4 and V5, respectively).

WEOM	Volatile fatty acid				
	Acetic acid ($mg\,L^{-1}$)	Propionic acid ($mg\,L^{-1}$)	*n*-Butyric acid ($mg\,L^{-1}$)	iso-Butyric acid ($mg\,L^{-1}$)	*n*-Valeric acid ($mg\,L^{-1}$)
Initial substrates					
FSS	96.7 ± 3.2	23.8 ± 1.4	14.4 ± 0.4	5.8 ± 0.2	10.5 ± 0.1
FCD	25.7 ± 1.1	3.8 ± 0.2	0.4 ± 0.0	ND[a]	ND
Vermicomposting					
V1	16.9 ± 0.6	1.7 ± 0.1	ND	ND	ND
V2	10.9 ± 0.5	0.1 ± 0.0	ND	ND	ND
V3	7.3 ± 0.3	ND	ND	ND	ND
V4	3.7 ± 0.2	ND	ND	ND	ND
V5	2.4 ± 0.1	ND	ND	ND	ND

[a] ND, not detected.

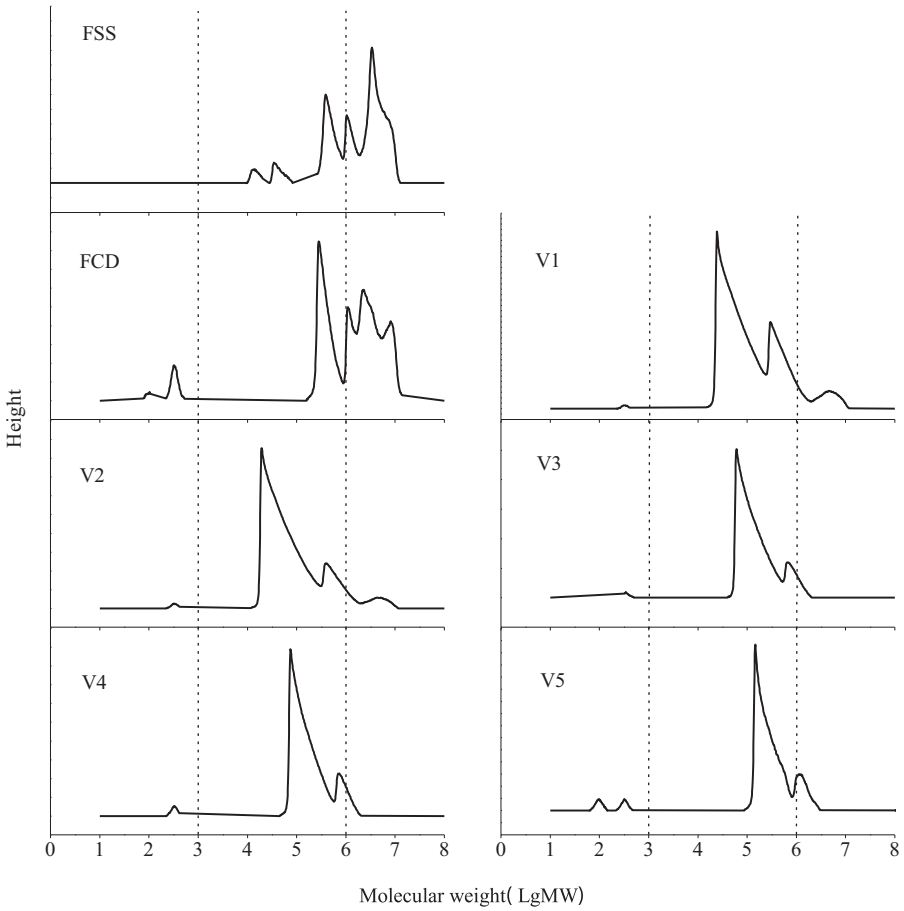

Fig. 1. Molecular weight distribution of water-extracted organic matter from fresh sewage sludge and cow dung (FSS and FCD), and the vermicomposts of different-proportion mixtures of sewage sludge and cow dung (100% + 0%, 70% + 30%, 50% + 50%, 30% + 70%, and 0% + 100%; V1, V2, V3, V4 and V5, respectively).

new components of WEOM, which seemed to be the stabilized fraction of WEOM [22].

The percentage of the fraction with molecular weights between 10^3 and 10^6 Da tended to decrease from V1 to V5, while the fractions with molecular weight less than 10^3 Da increased gradually, possibly ascribed to the enhancement of polycondensation of the fraction as the proportion of cow dung increased. Polycondensation of organic matter caused the aggregation of macromolecules and the generation of small molecules (such as H_2O, HX, and amines). The percentage of the fractions with molecular weights more than 10^6 Da initially decreased, and then increased from V1 to V5, likely because the degradation of labile macromolecule organic matter slowed and the aromaticity and polycondensation of the fraction with molecular weight between 10^3 and 10^6 Da increased as the proportion of cow dung increased from V1 to V5.

3.4. ^1H NMR spectra

The ^1H NMR spectra of WEOM from the initial substrates and vermicomposts are presented in Fig. 2, while the distribution of H moieties was outlined in Table 4. Two main characteristic areas that were observed are 0–3 ppm – terminal methyl groups of methylene chains, protons on methyl groups of highly branched aliphatic structure and protons on aliphatic carbon which are two

Table 3
Molecular weight of water-extracted organic matter from fresh sewage sludge and cow dung (FSS and FCD), and the vermicomposts of different-proportion mixtures of sewage sludge and cow dung (100% + 0%, 70% + 30%, 50% + 50%, 30% + 70%, and 0% + 100%; V1, V2, V3, V4 and V5, respectively).

WEOM (%)	Molecular weight ($\times 10^3$ Da)		
	<1	1–1000	>1000
Initial substrates			
FSS	0.01	34.95	65.04
FCD	5.17	34.76	60.08
Vermicomposting			
V1	0.35	90.48	9.18
V2	0.60	92.39	7.01
V3	0.76	95.34	3.90
V4	1.73	93.76	4.51
V5	4.99	80.49	14.52

Table 4
Distribution of hydrogen moieties in water-extracted organic matter (WEOM) fractions from fresh sewage sludge and cow dung (FSS and FCD), and the vermicomposts of different-proportion mixtures of sewage sludge and cow dung (100% + 0%, 70% + 30%, 50% + 50%, 30% + 70%, and 0% + 100%; V1, V2, V3, V4 and V5, respectively) according to liquid-state ^1H NMR spectroscopy.

WEOM	H moieties (%)	
	Aliphatic H (0.00–3.00 ppm)	H associated with O-containing functionalities (3.00– 4.25 ppm)
Initial substrates		
FSS	78.7	21.3
FCD	39.8	60.2
Vermicomposting		
V1	38.6	61.4
V2	36.8	63.2
V3	23.7	76.3
V4	29.2	70.9
V5	18.5	81.5

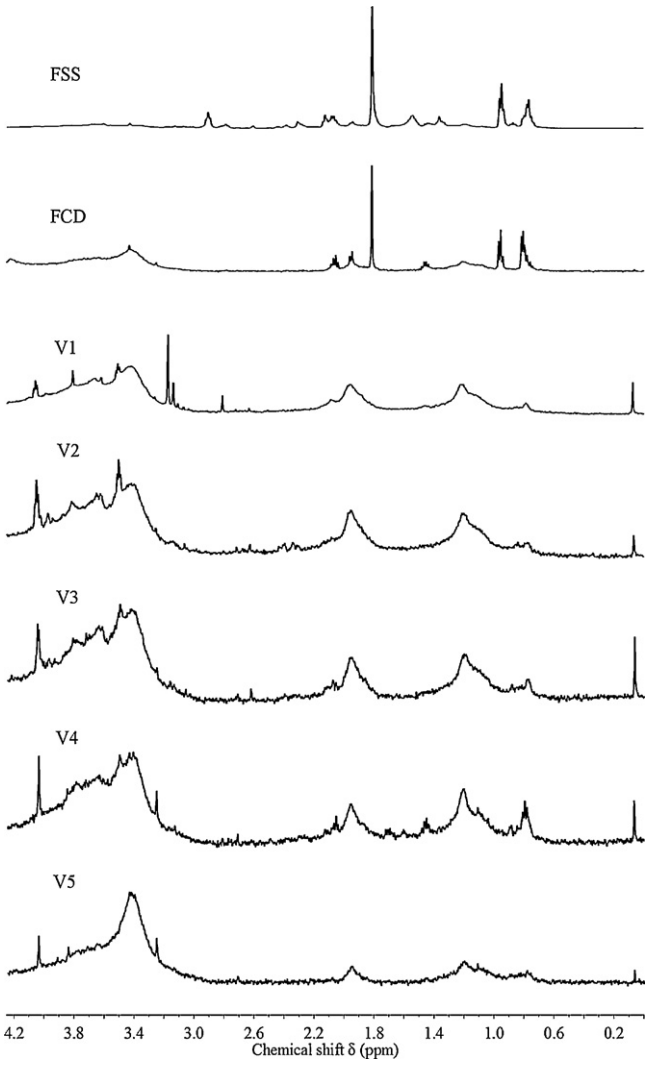

Fig. 2. ^{1}H NMR spectra of water-extracted organic matter from fresh sewage sludge and cow dung (FSS and FCD), and the vermicomposts of different-proportion mixtures of sewage sludge and cow dung (100% + 0%, 70% + 30%, 50% + 50%, 30% + 70%, and 0% + 100%; V1, V2, V3, V4 and V5, respectively).

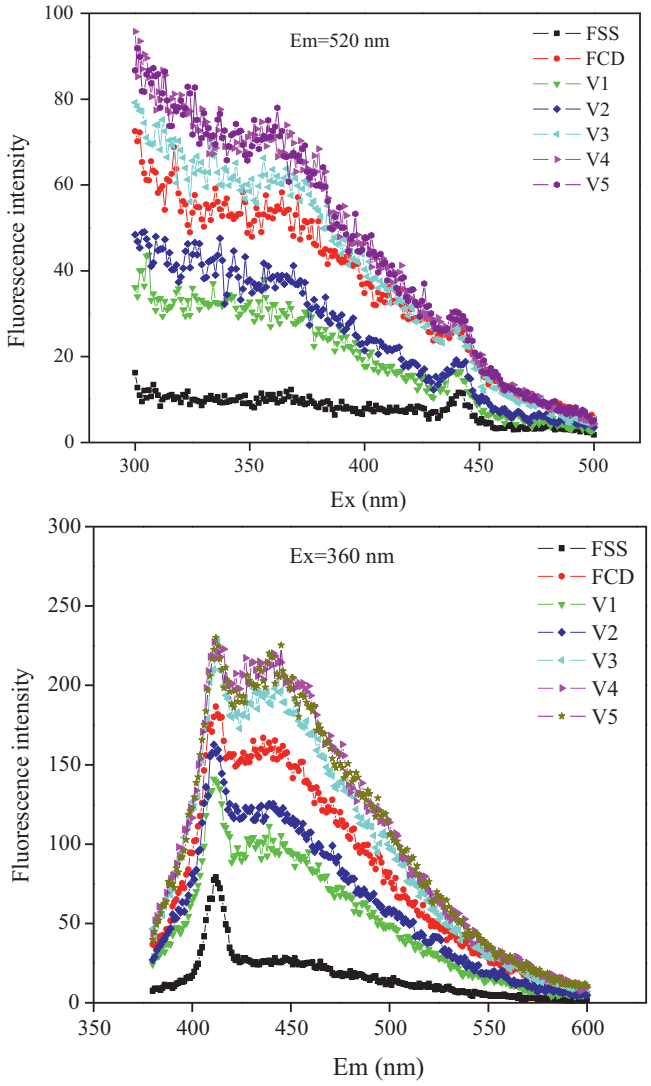

Fig. 3. Fluorescence excitation (E_x) and emission (E_m) spectra of water-extracted organic matter from fresh sewage sludge and cow dung (FSS and FCD), and the vermicomposts of different-proportion mixtures of sewage sludge and cow dung (100% + 0%, 70% + 30%, 50% + 50%, 30% + 70%, and 0% + 100%; V1, V2, V3, V4 and V5, respectively).

or more carbons away from the aromatic rings or polar functional groups [24,39] and 3–6.5 ppm – protons on carbons attached to O or N heteroatoms [39]. The results showed that vermicomposting considerably changed the ^{1}H NMR spectra and the H moieties distribution on the substrates, and the changes tended to be more and more marked from V1 to V5 with the increasing proportion of cow dung in the mixtures.

After vermicomposting process, the proportion of H moieties at 0.00–3.00 ppm declined. The signals at 0.00–3.00 ppm were associated with low molecular-weight substances such as acetate (1.93 ppm), propionate (1.23, 2.38 ppm), lactate (1.33 ppm), and succinate (2.43 ppm) [22,24]. Thus, vermicomposting led to the decomposition of low molecular-weight materials, such as acetate and propionate, corresponding to the previous data suggesting that the VFA contents in vermicomposts were lower than that noted in the initial substrates.

The percentage of H moieties at 3.00–4.25 ppm in the substrates increased remarkably after vermicomposting, especially in V5. The main contributions giving rise to ^{1}H resonance signals at 3.75–3.92 ppm were likely to arise from CHOH and CH_2OH functional groups which may indicate the presence of a methoxyphenylpropyl repeating unit which typically occurs in lignin and lignin-like matter [24,40,41]. Thus, the WEOM from the final vermicomposts seemed to be dominated by the lignin and lignin-like substances, especially in V5. The observations confirmed and complemented further previous results that the molecular weight of WEOM tended towards homogeneity after vermicomposting.

3.5. Fluorescence spectra

The fluorescence excitation and emission spectra of WEOM from all substrates are shown in Fig. 3. The fluorescence intensities of excitation and emission spectra in FSS were lower than that noted in FCD. After vermicomposting, the fluorescence intensities of two spectra in all substrates increased significantly. According to Chen et al. [42], the fluorescence intensities of excitation spectra with the range 300–500 nm at a fixed emission wavelength of 520 nm and emission spectra with the range 380–550 nm at a fixed excitation wavelength of 360 nm were related to the content of humic acid-like (HAL) fraction and therefore our data indicate likely formation of these compounds after vermicomposting, especially in V5 samples. Because the amount and quality of HAL components in the

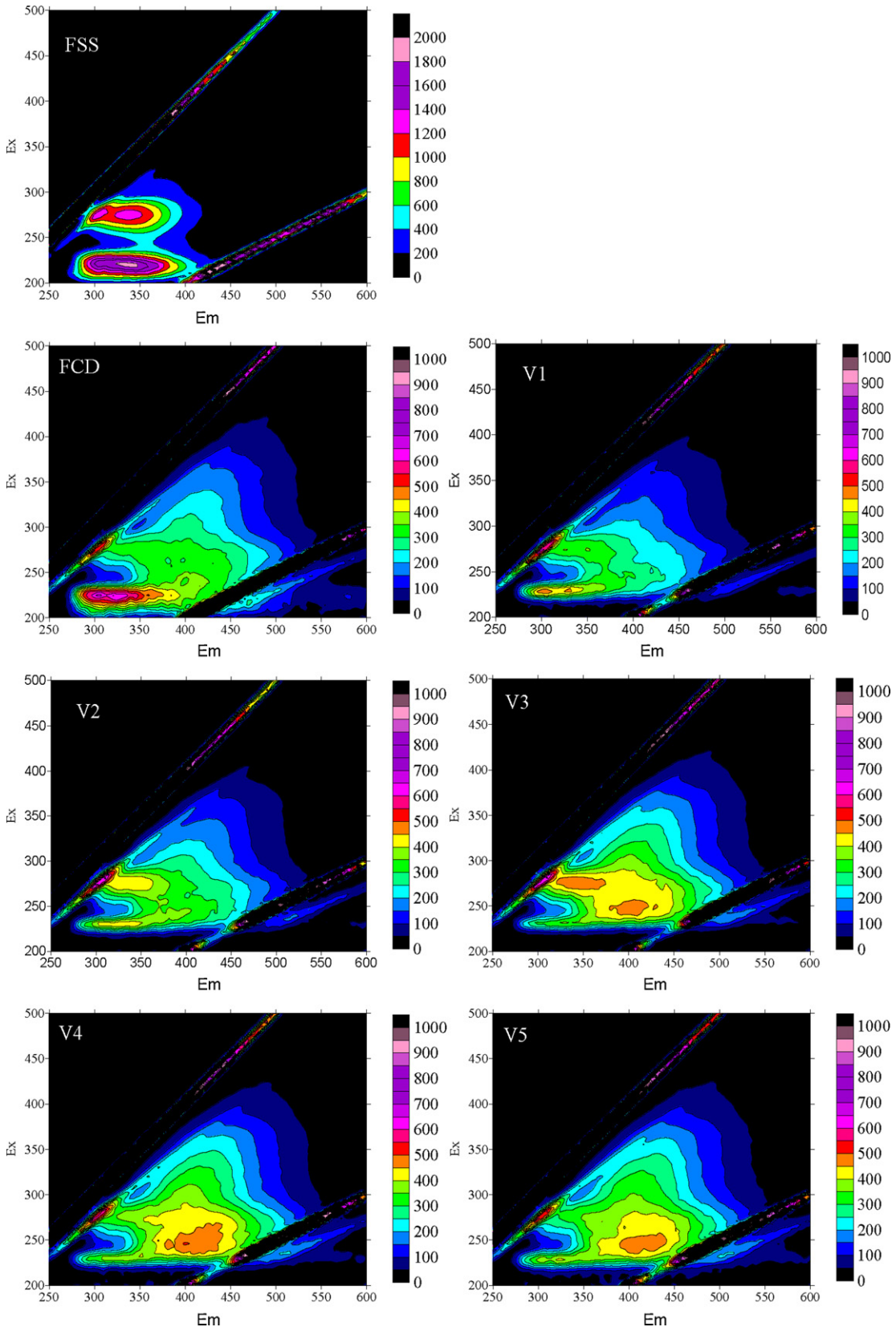

Fig. 4. EEM spectra of water-extracted organic matter from fresh sewage sludge and cow dung (FSS and FCD), and the vermicomposts of different-proportion mixtures of sewage sludge and cow dung (100% + 0%, 70% + 30%, 50% + 50%, 30% + 70%, and 0% + 100%; V1, V2, V3, V4 and V5, respectively).

Table 5
E_x/E_m maxima of water-extracted organic matter from fresh sewage sludge and cow dung (FSS-HAL and FCD-HAL), and vermicomposts of different-proportion mixtures of sewage sludge and cow dung (100% + 0%, 70% + 30%, 50% + 50%, 30% + 70%, and 0% + 100%; V1, V2, V3, V4 and V5, respectively).

Sample	Peak 1		Peak 2		Peak 3	
	E_x/E_m[a]	SFI[b]	E_x/E_m	SFI	E_x/E_m	SFI
Initial substrates						
FSS	220/330	1894	275/340	1302	–	–
FCD	225/320	660.8	275/360	354.2	230/365	474.3
Vermicomposting						
V1	230/330	509.8	275/350	361.6	245/380	323.8
V2	230/330	466.5	275/350	449.0	235/375	382.2
V3	230/330	374.8	275/390	448.3	250/405	490.0
V4	230/340	410.	–	–	240/400	509.9
V5	230/325	427.2	–	–	245/410	490.9

[a] E_x/E_m represented the excitation/emission wavelength pairs.
[b] SFI referred to the specific fluorescence intensity.

vermicomposts are considered important indicators of biological maturity and chemical stability [2,43], an increase in HAL fraction of WEOM indicated a progression or transformation to more chemically stable sewage sludge.

Fluorescence EEM spectra of WEOM from the initial substrates and vermicomposts are shown in Fig. 4. Both FSS and FCD were characterized by several fluorophores, but had their own excitation/emission wavelength pairs (EEWPs) and specific fluorescence intensity (SFI) (Table 5). FSS had two main peaks (peaks 1 and 2), while three main peaks (peaks 1–3) were found in FCD. According to Chen et al. [42], peak 1 belongs to an aromatic protein region, and could include tryptophan and BOD_5, peak 2 pertains to soluble microbial by-product-like materials and peak 3 falls in the region related to the amount of fulvic acid-like compounds.

After vermicomposting, the SFI of peak 1 tended to decrease, while that of peak 3 tended to increase from V1 to V5 (Table 5). Additionally, the peak positions (peaks 1 and 3) in the fluorescence spectra of the substrates shifted markedly towards longer wavelengths (Fig. 3). Chen et al. [44] reported that the peak position of fluorescence emission shifted to a longer wavelength with an increasing content of aromaticity and polycondensation of humic materials, findings in accord with $SUVA_{254}$ results.

Thus, vermicomposting led to the decrease of protein-like groups, and the increase of fulvic acid-like and HAL materials in the WEOM, corresponding to the previous findings in DOC and SUV_{254}, substantial indications of enhanced maturity and bio-stability of sewage sludge.

4. Conclusion

Vermicomposting of sewage sludge enriched with cow dung resulted in a decrease in DOC, DCOD and VFA contents, and C/N ratio of WEOM, and an increase in $SVUA_{254}$ and DTN levels. Additionally, with the increasing proportion of cow dung in the substrates DOC loss and aromaticity in vermicompost derived markedly increased, indicating the addition of cow dung improved the processes for maturation and stabilization of sewage sludge.

Vermicomposting homogenized the molecular weight of WEOM from the substrates. After vermicomposting, the WEOM from the substrates was characterized by small aliphatic character and protein-like groups, and high O-containing groups and fulvic acid-like and HAL materials. In particular, the changes in WEOM from vermicompost of cow dung alone were the most marked.

In sum, the findings implied that vermicomposting promoted the degradation and transformation of labile WEOM into biologically stable substances, and thus improved the bio-stabilization of sewage sludge particularly after enrichment with cow dung. Additionally, analyses following water-extracted organic matter might be effective in assessing the biological maturity and chemical stability of vermicompost.

Acknowledgement

The research was supported by The National Natural Science Foundation of China (51109161), The fundamental research funds for the central universities (0400219187), The National Spark Program of China (2010GA680004), and The Young Cadre Teachers of Tongji University Project (2008KJ021). The Program for New Century Excellent Talent in University (NCET-08-0404) and the Excellent Doctoral Dissertation Nationwide Foundation (200756) are also acknowledged. The authors would thank Kevin Sellner for supplying the suggestions about English expression and writing of this paper.

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