



## Compositional and functional features of humic acid-like fractions from vermicomposting of sewage sludge and cow dung

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### ABSTRACT

The chemical changes occurring in five different substrates of sewage sludge spiked with different proportions of cow dung after vermicomposting with *Eisenia foetida* for 90 days were investigated. Their humic acid-like (HAL) fractions were isolated to determine the elemental and functional composition, and structural and functional characteristics using ultraviolet/visible, Fourier transform infrared (FT-IR) and fluorescence spectroscopies and scanning electron microscopy. After vermicomposting, the total organic C and C/N ratio decreased, and the total extractable C and humic acid (HA) C increased in all substrates. In the HAL fractions, the C and H contents, C/N and C/O and aliphatic structures, proteinaceous components and carbohydrates decreased, while the O and N and acidic functional group contents and C/H ratio, aromaticity and polycondensation structures increased. Further, the results suggest that the addition of cow dung to sewage sludge could improve the quality of organic matter humification of the substrates. The structures of HAL fractions in vermicomposts resembled those typical of soil HA, especially the vermicompost of cow dung alone. Scanning electron microscopy showed the microstructure of HAL fraction in final product became close-grained and lumpy. Overall results indicate that vermicomposting was an efficient technology for promoting organic matter (OM) humification in sewage sludge and cow dung alone, as well as in mixtures of both materials, improving their quality and environmental safety as a soil OM resource for utilization as soil amendments.

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### 1. Introduction

In China, the production of sewage sludge from the wastewater treatment plants (WWTPs) has been increasing dramatically due to expanding urbanization, the demand for better quality water and the imposition of stricter environmental laws [1]. Treatment and disposal of sewage sludge is one of the most critical environmental issues today [2,3], with costs for these processes accounting for about half, even up to 60%, of the total wastewater treatment expense [4–9]. Thus, identifying simple technologies for sludge reduction and stabilization with low operation and maintenance costs is a high priority [10,11].

The potential use of earthworms to break down and manage sewage sludge began in the late 1970s [12], which have been termed vermicomposting or vermistabilization [13,14]. Vermicomposting, involving the joint action of earthworms and microorganisms [15–17], considerably improved the decomposition and stabilization of sludge. Moreover, the end products in the

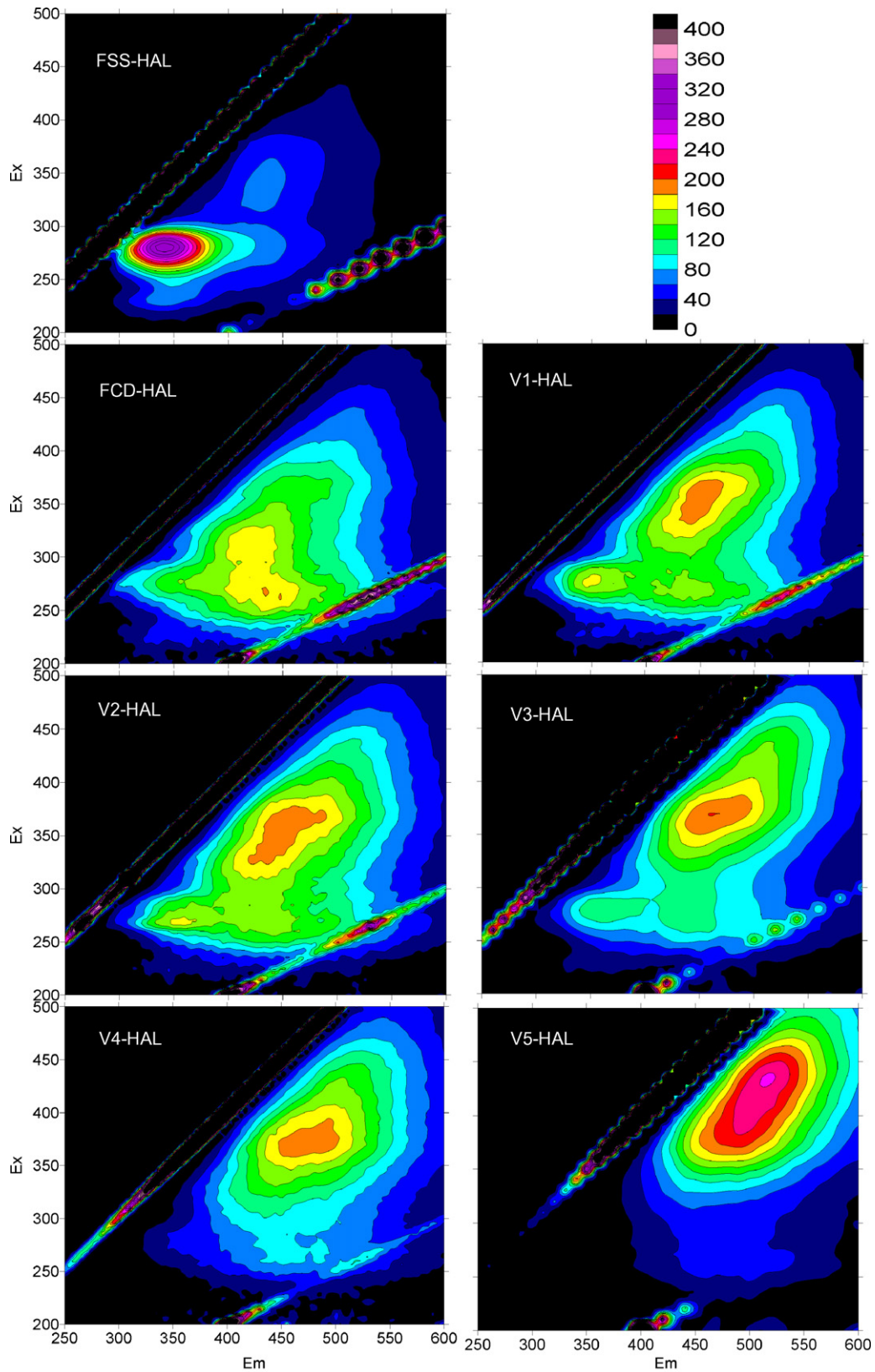
vermicomposting process were found to have higher N and P contents [18,19] and lower human pathogen [3,20], yielding an organic amendment or a soil conditioner. Vermicompost, as a soil organic amendment, could improve the physical, chemical, and microbial properties of the soil, and stimulate plant growth [21–26].

Previous studies on vermicomposting focused on the stabilization of sewage sludge, evaluation of vermicompost as soil organic amendment or fertilizer, and earthworm population dynamics [3,9,14,27]. To date, the influence of vermicomposting on chemical features of the humic acid-like (HAL) fractions in sewage sludge, and added enrichments such as cow dung, has not been fully studied.

The amount and quality of HAL components in composts and vermicomposts were considered as important indicators of their biological maturity and chemical stability, and a guarantee for safe and successful performance in soil [16,28,29]. Enhanced organic matter humification of winery and distillery wastes was found in vermicomposting for these initial residues [16], as well as for cattle manure mixed with olive pomace [30], sugar industry wastes [31], and composted olive mill wastes [32]. The features of HAL fractions have been studied using advanced physico-chemical techniques such as Fourier transform infrared (FT-IR), fluorescence, nuclear magnetic resonance ( $^{13}\text{C}$ ) and UV spectroscopies, thermal analysis

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**Fig. 1.** Fluorescence excitation–emission matrix spectra (as contour maps) of humic acid-like fractions extracted 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-HAL, V2-HAL, V3-HAL, V4-HAL and V5-HAL, respectively).

**Table 1**  
Chemical properties and humification parameters of 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).

	pH	EC <sup>a</sup> (μs/cm)	TOC <sup>b</sup> (g/kg)	Total N (g/kg)	TEC <sup>c</sup> (g/kg)	FAC <sup>d</sup> (g/kg)	HAC <sup>e</sup> (g/kg)	C/N	HR <sup>f</sup> (%)	HI <sup>g</sup> (%)
Initial substrates										
FSS	6.77 ± 0.01	1071 ± 21	368.5 ± 2.0	35.80 ± 0.3	40.0 ± 1.1	35.6 ± 0.5	4.4	10.29	11	1
FCD	8.67 ± 0.01	680 ± 6	386.1 ± 1.2	22.50 ± 0.6	53.0 ± 0.8	36.7 ± 0.4	16.3	17.16	14	4
Vermicomposting										
V1	5.49 ± 0.01	3990 ± 47	253.2 ± 3.8	35.58 ± 0.7	67.6 ± 1.2	44.7 ± 0.3	22.9	7.11	27	9
V2	5.85 ± 0.00	3750 ± 29	246.2 ± 3.1	30.03 ± 0.7	75.2 ± 0.5	35.0 ± 1.2	40.2	8.20	31	16
V3	6.12 ± 0.01	3320 ± 41	239.8 ± 2.7	27.36 ± 0.4	88.6 ± 1.0	32.9 ± 0.8	55.7	8.76	37	23
V4	6.88 ± 0.00	2610 ± 32	228.0 ± 3.5	25.58 ± 0.3	94.4 ± 0.6	33.2 ± 0.2	61.2	8.91	41	27
V5	7.33 ± 0.02	1922 ± 11	221.0 ± 1.1	23.28 ± 0.1	114.3 ± 0.9	29.6 ± 1.0	84.7	9.49	52	38

<sup>a</sup> Electrical conductivity.

<sup>b</sup> Total organic C.

<sup>c</sup> Total extractable C.

<sup>d</sup> Fulvic acid C.

<sup>e</sup> Humic acid C.

<sup>f</sup> Humification rate.

<sup>g</sup> Humification index.

**Table 2**  
Elemental composition and atomic ratios (±standard errors of three laboratory replicates, moisture- and ash-free) of humic acid-like fractions extracted 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-HAL, V2-HAL, V3-HAL, V4-HAL and V5-HAL, respectively).

Origin of humic acids	C (%)	O (%)	H (%)	N (%)	S (%)	Atomic ratio		
						C/H	C/N	C/O
Initial substrates								
FSS-HAL	56.89 ± 0.17	29.58 ± 0.09	8.32 ± 0.05	4.53 ± 0.05	0.68 ± 0.01	0.57	14.65	2.56
FCD-HAL	61.13 ± 0.21	24.76 ± 0.14	9.27 ± 0.06	4.12 ± 0.07	0.72 ± 0.02	0.55	17.31	3.29
Vermicomposting								
V1-HAL	51.09 ± 0.31	35.84 ± 0.09	7.26 ± 0.07	5.03 ± 0.07	0.78 ± 0.01	0.59	11.85	1.90
V2-HAL	52.25 ± 0.15	34.57 ± 0.11	7.25 ± 0.03	5.04 ± 0.05	0.89 ± 0.03	0.60	12.09	2.02
V3-HAL	52.47 ± 0.25	34.49 ± 0.06	7.09 ± 0.09	5.14 ± 0.03	0.81 ± 0.04	0.62	11.91	2.03
V4-HAL	52.77 ± 0.09	34.26 ± 0.12	6.87 ± 0.03	5.25 ± 0.04	0.85 ± 0.01	0.64	11.73	2.05
V5-HAL	51.53 ± 0.22	35.64 ± 0.14	6.55 ± 0.08	5.37 ± 0.06	0.91 ± 0.02	0.66	11.20	1.93

**Table 3**  
Acidic functional group contents (±standard errors of three laboratory replicates, moisture- and ash-free) and E4/E6 ratio of humic acid-like fractions (HAL) extracted 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-HAL, V2-HAL, V3-HAL, V4-HAL and V5-HAL, respectively).

Origin of humic acids	Total acidity (mmol/g)	COOH (mmol/g)	Phenolic OH (mmol/g)	E4/E6 ratio
Initial substrates				
FSS-HAL	2.27	1.30 ± 0.02	0.97 ± 0.03	9.12 ± 0.42
FCD-HAL	2.88	1.76 ± 0.03	1.12 ± 0.01	7.67 ± 0.29
Vermicomposting				
V1-HAL	3.18	1.89 ± 0.04	1.29 ± 0.03	4.60 ± 0.11
V2-HAL	3.47	2.11 ± 0.02	1.36 ± 0.02	4.33 ± 0.23
V3-HAL	3.71	2.27 ± 0.06	1.44 ± 0.04	4.08 ± 0.46
V4-HAL	4.03	2.45 ± 0.04	1.58 ± 0.01	3.62 ± 0.35
V5-HAL	4.39	2.68 ± 0.01	1.71 ± 0.05	3.23 ± 0.49

**Table 4**  
Excitation–emission matrix maxima of humic acid-like fractions extracted 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-HAL, V2-HAL, V3-HAL, V4-HAL and V5-HAL, respectively).

Sample	Peak 1		Peak 2		Peak 3	
	Ex/Em <sup>a</sup>	SFI <sup>b</sup>	Ex/Em	SFI	Ex/Em	SFI
Initial substrates						
FSS-HAL	280/340	341			340/435	67
FCD-HAL	275/365	148.2	265/435	182.9	325/440	168.1
Vermicompost						
V1-HAL	275/345	170	270/435	158.3	360/450	194.1
V2-HAL	270/355	167.8	270/440	158.9	365/450	201.1
V3-HAL	280/360	119.8	270/445	113.3	370/465	203.6
V4-HAL			280/450	95.9	370/490	198.3
V5-HAL					430/515	244

<sup>a</sup> Excitation/emission wavelength pairs.

<sup>b</sup> Specific fluorescence intensity.

**Table 5**  
Selected infrared indicator bands in humic acid-like fractions.

Wave number (cm <sup>-1</sup> )	Vibration	Functional group or component
3367–3300	O–H stretching N–H stretching	Bonded and non bonded hydroxyl groups and water Various functional groups
2931–2925, 2854	C–H stretching	Aliphatic methylene group
1651–1655	C=O stretching C=C stretching	Amide I, carboxylates Aromatic ring modes, alkenes
1570–1540	N–H in plane	Amides II
1512–1511	Aromatic skeletal	Lignin
1458–1456	C–H stretching	Aliphatic group
1421–1417	COO <sup>-</sup> stretching C=N stretching	Carboxylic acids Amide III
1320	C–N stretching	Aromatic primary and secondary amines
1260–1200	C–O stretching, O–H deformation	Carboxylic acids
1130	C–O stretching	Aryl ethers and phenols
1080–1030	C–OH stretching C–O stretching	Aliphatic OH Polysaccharides or polysaccharide-like substances
850–750	Si–O NH <sub>2</sub> out of plane	Silicate impurities Primary amine group

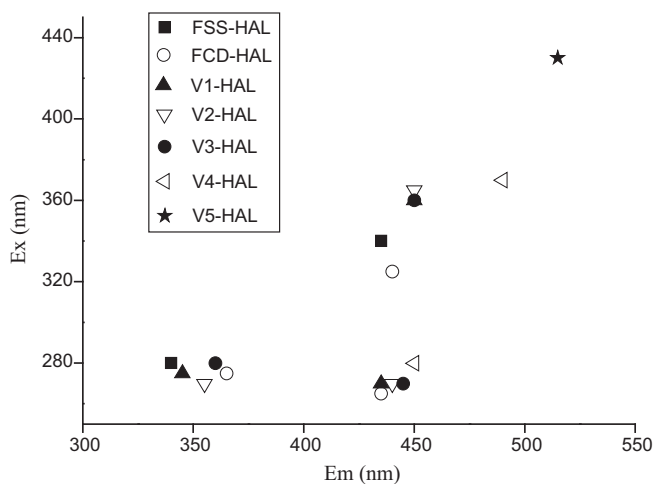
and thermochemolysis–gas chromatography–mass spectrometry analysis [16,32–34]. However, few papers reported microstructure of the HAL fraction in sewage sludge enriched with additives common to China (such as cow dung) using scanning electron microscopy (SEM).

The information is limited regarding the suitability of vermicomposting for transforming organic matter from sewage sludge and the cow dung additive into humic substances, and the subsequent quality of the vermicomposts as soil organic amendments. Thus, the objectives of the work are to investigate: (a) the chemical changes in vermicomposting process of sewage sludge mixed with cow dung; and (b) the compositional, functional and structural features of the HAL fractions extracted from the initial substrates and their vermicomposts using UV and FT-IR and fluorescence spectroscopies and SEM.

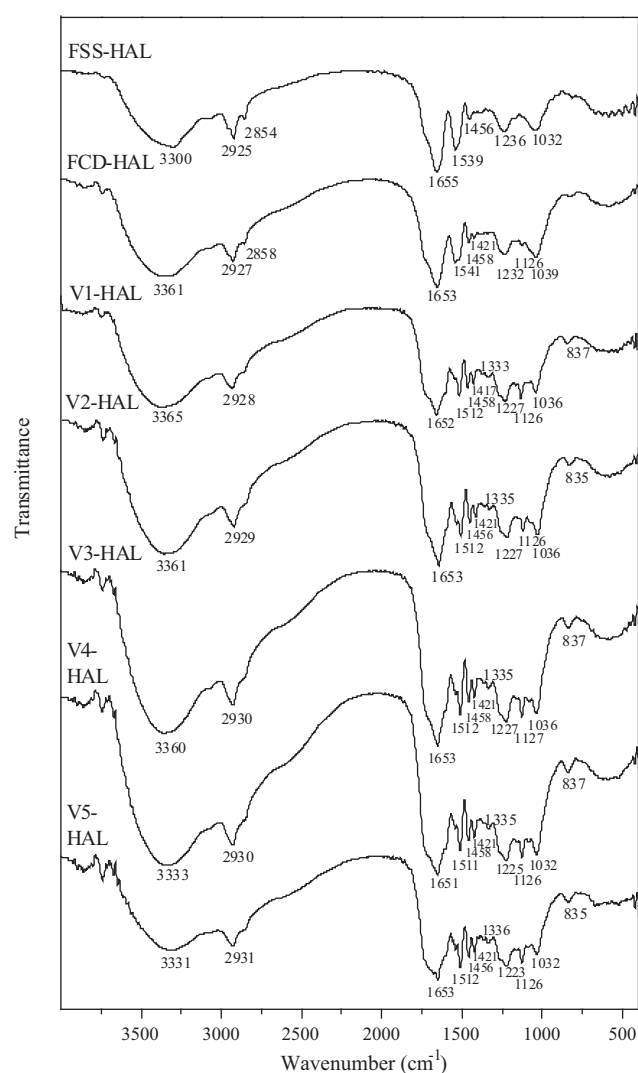
## 2. Materials and methods

### 2.1. Substrates and vermicomposting process

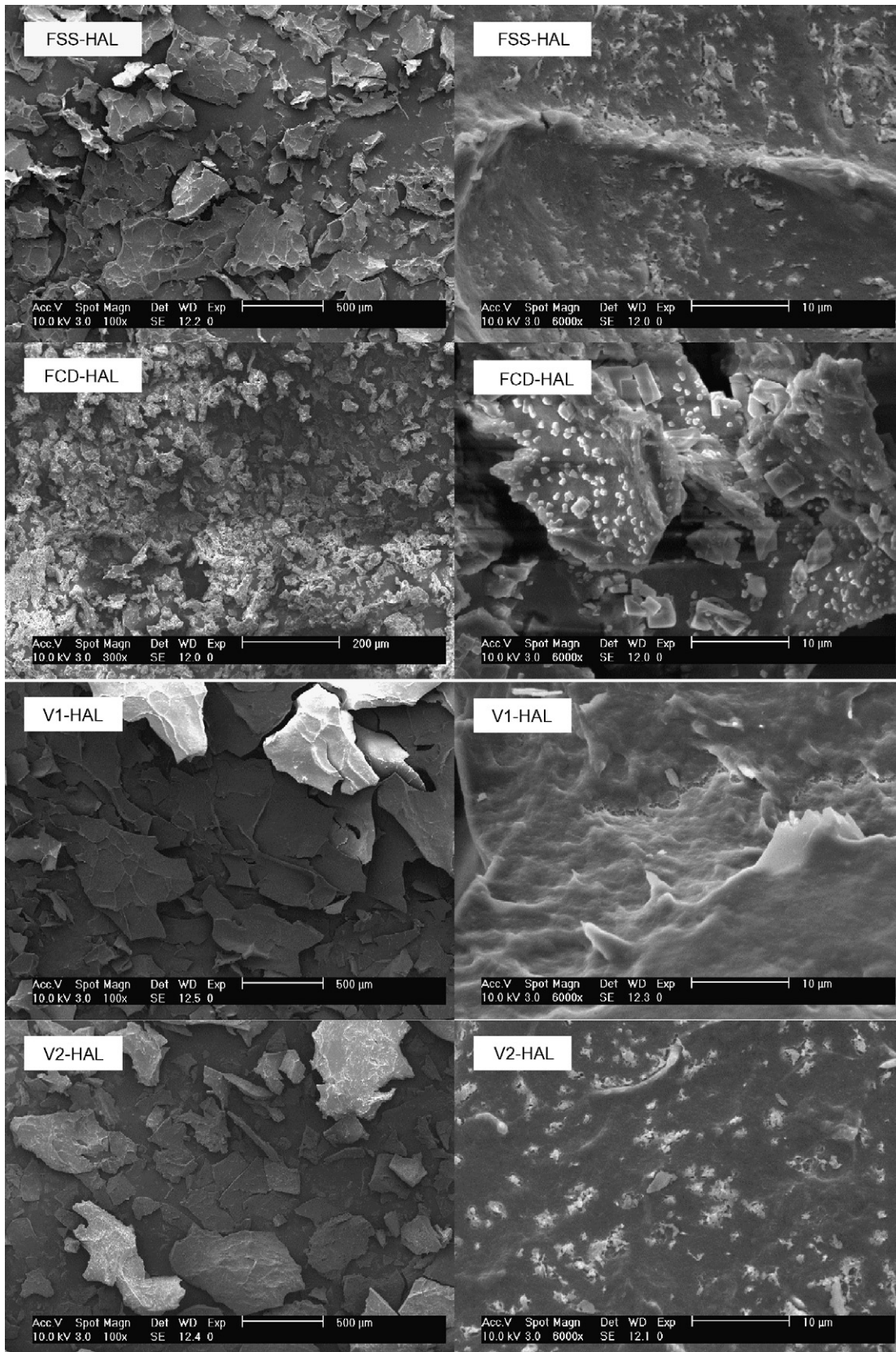
Fresh sewage sludge (FSS) was procured from the dumping site of a domestic WWTP (Quyong WWTP, Shanghai, China). The water content of FSS was about 75–85%. The wastewater treatment capac-



**Fig. 2.** Distribution of excitation–emission matrix maxima of humic acid-like fractions extracted 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-HAL, V2-HAL, V3-HAL, V4-HAL and V5-HAL, respectively).



**Fig. 3.** Fourier transform infrared spectra of humic acid-like fractions extracted 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-HAL, V2-HAL, V3-HAL, V4-HAL and V5-HAL, respectively).



**Fig. 4.** Scanning electron microscopy of humic acid-like fractions extracted 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-HAL, V2-HAL, V3-HAL, V4-HAL and V5-HAL, respectively).

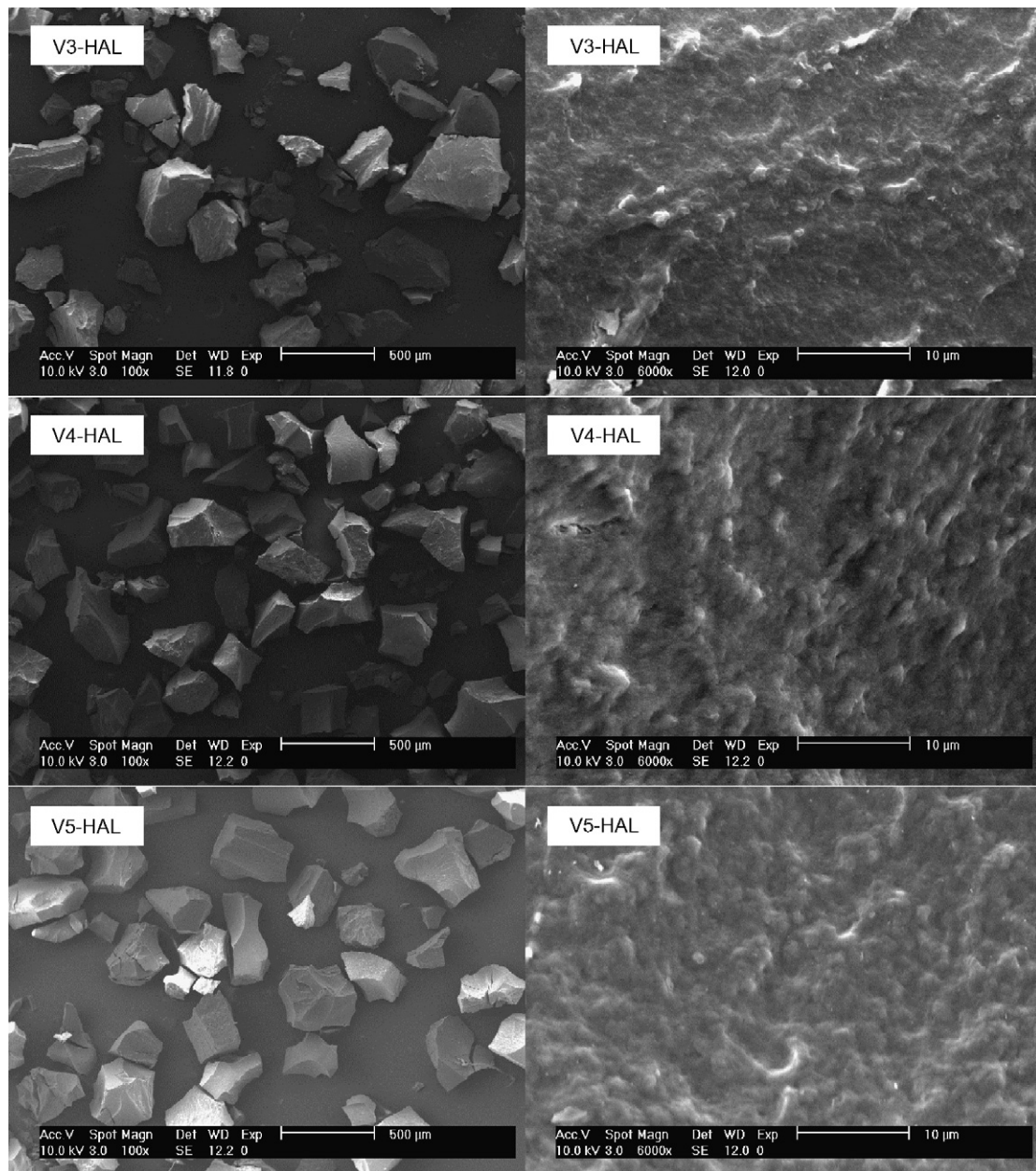


Fig. 4. (Continued.)

ity of this plant was  $60,000 \text{ m}^3 \text{ d}^{-1}$ . Fresh cow dung (FCD) was obtained from a cow farm in the suburb of Huangpu district, Shanghai, China. The FSS and FCD were dried in direct sunlight for two weeks, with periodic turning. Young non-clitellated earthworms *Eisenia foetida* were randomly picked from several stock cultures maintained in the laboratory with cow dung as culturing substrate.

Six circular plastic containers (15 cm diameter  $\times$  14 cm depth) were filled with 100 g feed mixture (dry weight) containing different proportions 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, 10 non-clitellated earthworms, weighing 200–250 mg live weight, were introduced into 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 temper-

ature ( $25 \pm 1^\circ \text{C}$ ). All containers were vermicomposted for 90 days followed by separation of earthworms, cocoons and straw. Vermicomposts were collected from each container for further analyses.

## 2.2. Analysis of substrates

The initial substrates (FSS and FCD) and vermicomposts were air-dried at room temperature, and then crushed to pass through a 0.2 mm sieve. Thereafter, the pH and electrical conductivity (EC) were measured in 1/20 (W/V) aqueous solutions. Total organic carbon (TOC) and total nitrogen (TN) were measured by a TOC-VCPN analyzer (Shimadzu, Japan). The total extractable C (TEC), humic acid-like C (HAC), and fulvic acid-like C (FAC) contents were recorded according to the methods proposed by Romero et al. [16]. The humification ratio (HR) was obtained as  $\text{HR}\% = 100 \times \text{TEC}/\text{TOC}$ , and the humification index (HI) as  $\text{HI}\% = 100 \times \text{HAC}/\text{TOC}$  [35].

### 2.3. Isolation of humic acids

The HAL fractions were isolated from the initial substrates and vermicomposts according to a conventional procedure [36]. In short, 1.0 g of each sample, previously air-dried and 0.5-mm sieved, were extracted by 10 mL 0.1 mol L<sup>-1</sup> Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> and 10 mL 0.5 mol L<sup>-1</sup> NaOH. The mixtures were vibrated on a DKY II rotary shaking platform (Duke Equipment Automation Co., LTD., Shanghai, China) in capped plastic bottles for 24 h at room temperature (RT, 25 °C). The supernatant solutions were then separated from the residues by centrifugation at 9600 × g for 30 min. The combined alkaline supernatants were acidified to pH 1 with 6 mol L<sup>-1</sup> HCl, allowed to stand for 24 h at 4 °C, and then centrifuged at 10,000 × g for 30 min. The HAL fractions were purified in 5 mL volume of 0.3 mol L<sup>-1</sup> KCl and 5 mL of 0.2 mol L<sup>-1</sup> KOH, and then washed several times with water until the last rinse yielded a negative chloride test with silver nitrate [36]. The residues were freeze-dried and stored in plastic vials placed in a desiccator containing P<sub>2</sub>O<sub>5</sub>. Moisture and ash contents were measured by heating the HAL fraction overnight at 105 °C and for 2 h at 550 °C, respectively.

### 2.4. Humic acid analyses

The C, H, N and S contents of HAL fractions were measured in triplicate using an elemental analyzer Vario EL III (German). Oxygen content was calculated by difference: O% = 100 – (C + H + N + S)%.

Carboxylic group and phenolic hydroxyl group content were determined using the method proposed by Ritchie and Perdue [37]. Total acidity was estimated by addition of both contents.

The E4/E6 ratios were given as the ratio of absorbances measured at 465 and 665 nm on solutions of 3.0 mg of each HAL fraction in 10 mL of 0.05 mol L<sup>-1</sup> NaHCO<sub>3</sub>, with the pH adjusted to 8.3 with 0.02 mol L<sup>-1</sup> NaOH [38–40].

Fluorescence excitation–emission matrix (EEM) spectra were obtained on aqueous solutions of HAL fractions at a concentration of 100 mg L<sup>-1</sup> after overnight equilibration at 25 °C, and adjustment to pH 8 with 0.05 mol L<sup>-1</sup> NaOH, using F-4600 Fluorescence Spectrophotometers (Hitachi, Japan). Emission and excitation slits were set at a 5-nm band width, and a scan speed of 12,000 nm min<sup>-1</sup> was selected for both monochromators. The EEM spectra were recorded by scanning the emission wavelength over the range 250–600 nm, while the excitation wavelength was increased sequentially from 200 to 500 nm. Surfer 8.0 software was used to analyze fluorescence spectral data.

The Fourier transform infrared (FT-IR) spectra of HAL fractions were recorded on pellets obtained by pressing under reduced pressure a mixture of about 1 mg of HAL sample and about 400 mg of dried KBr, spectrometry grade. A Nicolet 5700 FT-IR spectrophotometer (Madison, WI) and Origin 7.5 software were used to gain and analyze the spectra data.

The microstructures of HAL fractions were determined using an XL 30 Field Emission Gun Scanning Microscope (Philips, Netherlands).

## 3. Results and discussion

### 3.1. Chemical properties and humification parameters of substrates

Chemical characteristics and humification parameters of the initial substrates and vermicomposts are displayed in Table 1. Compared to FCD, FSS had lower pH values, TOC, TEC, FAC and HAC contents, C/N ratio, HR and HI values, and higher EC and TN contents.

Except TN and FAC contents, the vermicomposts in all the substrates had marked changes, but the final characteristics (chemical characteristics and humification parameters of vermicomposts) differed. After vermicomposting, pH values in all mixtures decreased compared to observations in the initial substrates, corroborating with the findings of other researchers (e.g., [27]). The pH decrease probably resulted from mineralization of nitrogen and phosphorus to nitrites/nitrates and orthophosphates, respectively, and bioconversion of the organic material into small organic acids [40]. Romero et al. [16] found that the pH in winery and distillery wastes increased after vermicomposting, which might be attributed to the mineralization of proteinaceous materials to yield alkaline ammonia and/or to loss of volatile acids.

In our experiments, EC values increased in all mixtures after vermicomposting, primarily attributed to degradation of organic matter and release of different mineral salts in available forms such as phosphate, ammonium, potassium, etc [41]. The pH and EC values differences among the substrates (from V1 to V5) were likely induced by the difference between electrolyze sources in FSS and FCD.

The TOC losses in final vermicomposts were approximated 31–43% of the initial TOC in the substrates, implying considerable net organic matter mineralization through the joint action of earthworms and microorganisms. After vermicomposting, the C/N ratio was lower, and the TEC and HAC contents and HR and HI values in all the substrates were higher than noted in the initial substrates, corresponding to results of other studies [16,30], implying that the stabilization of organic matter and enhancement of humification, especially in V5 samples, took place. Additionally, the mineralization and humification of organic matter in the substrates increased with the proportion of cow dung (from V1 to V5), suggesting that cow dung organic components might be more beneficial to degradation by earthworms and microorganisms than the organic constituents in the sewage sludge alone. This might explain why cow dung has often used to improve sewage sludge vermicomposting [27].

### 3.2. Chemical and structural characteristics of humic acid-like fraction

#### 3.2.1. Elemental and functional group composition and E4/E6 ratio

The elemental composition and atomic ratios of the HAL fractions are shown in Table 2, while the functional group composition and E4/E6 ratio are presented in Table 3. The initial elemental composition of the HAL fractions of FSS and FCD was different. With respect to FSS-HAL, FCD-HAL had larger C and H and S contents, C/N and C/O ratios, and smaller O and N contents and C/H ratio. Further, FSS-HAL displayed large E4/E6 ratio and small carboxyl and phenolic OH group contents, which led to a lower total acidity. The relatively high C and H contents and low O content are typical of HAL fractions isolated from decomposing plant residues and freshly formed, low-humified materials [29].

After vermicomposting, large O and N and acidic functional group contents and C/H ratio, and small C, H and S contents, C/N and C/O and E4/E6 ratios were found in all substrates. These results are in general agreement with those reported in vermicompost and compost of other wastes [16,30,32,42]. Thus, the HAL fractions in the vermicomposts revealed oxidation, dehydrogenation, and stabilization of organic matter (C loss), and the transformation of unsaturated structures to saturated structures [16].

The H content and E4/E6 ratio tended to decrease slightly from V1-HAL to those collected from V5-HAL, but the N and acidic functional group contents and C/H ratio showed opposite trends. The other elemental contents and atomic C/O ratios showed no persistent variation among the five vermicomposts. This indicated that

the addition of cow dung to sewage sludge had little influence on the elemental composition and atomic ratio of the vermicomposts in all substrates, likely because FCD-HAL had larger C- and smaller N-compounds and degree of oxidation than typified the sewage sludge.

The N content tended to increase slightly, implying the incorporation of N compounds of proteinaceous nature in HAs [36,42]. The changes of the E4/E6 ratio may have been inversely related to the particle size, molecular weight and humification degree of HAs [16,38]. Thus, the decrease of E4/E6 ratios measured for HAL fractions in the vermicomposts suggested enhancement of molecular association and humification degree.

In general, the results implied the chemical stability of HAL fractions in the final vermicomposts might increase their potential use as a soil amendment and agricultural fertilizer.

### 3.2.2. Fluorescence spectra

Fluorescence EEM spectra of the HAL fractions isolated from the initial substrates and vermicomposts are shown in Fig. 1. Both of FSS-HAL and FCD-HAL were characterized by several fluorophores, but had their own excitation/emission wavelength pairs (EEWPs) (Table 4). The main peak of FSS-HAL (peak 1) had shorter excitation and emission wavelengths, but higher specific fluorescence intensity (SFI) than that noted in FCD-HAL (Table 4). According to Chen et al. [43], peak 1 belongs to the aromatic protein region of the compound, might include tryptophan and BOD<sub>5</sub>, while Peak 2 and peak 3 fell in the region related with the amount of humic acid or fulvic acid. Thus, the results corresponded to the fact that the HAC and FAC contents and humification degree in FSS were lower than that noted in FCD (Table 1).

After vermicomposting, the peak positions in the fluorescence spectra of the substrates shifted markedly towards longer wavelengths (Fig. 2), in agreement with the previous findings in other vermicomposts and composts [16,30,32,42]. According to the Senesi et al. [44], FSS-HAL and FCD-HAL were related to the simple structural components of wide molecular heterogeneity and small molecular size, small levels of conjugated chromophores and low degrees of aromatic polycondensation and humification. On the contrary, the HAL fractions isolated from the vermicomposts featured an increase in molecular size, aromatic polycondensation, and larger degree of humification.

The EEWPs of main peaks from V1-HAL to V5-HAL increased gradually. In particular, the EEWPs of the main peak in V5-HAL shifted to 430 nm/515 nm. Chen et al. [45] reported that the peak position of fluorescence emission shifted to a longer wavelength with an increasing content of aromaticity and polycondensation of humic materials. The observations corresponded to the previous results that the humification degree of organic matter in the substrates increased with the proportion of cow dung and reached the highest levels in the vermicompost of cow dung alone (V5). Fluorescence features of HAL in vermicomposts resembled those typical of soil HAL [44].

In general, the fluorescence EEM spectra were consistent with results from the previous analyses and confirmed that aromaticity and polycondensation of the HAL fraction increased in vermicomposts, especially in V5-HAL (cow-dung alone).

### 3.2.3. FT-IR spectra

The FT-IR spectra of the HAL fraction in initial substrates and final vermicomposts are revealed in Fig. 3. The main absorption bands and corresponding assignments are outlined in Table 5, according to the literatures [16,30,39,46–48].

In general, FT-IR bands and peaks displayed a marked difference between the initial substrates and final vermicomposts. The main changes in FT-IR spectra of the HAL fractions from vermicomposts were: (1) a marked intensity decrease at about 2930 cm<sup>-1</sup>

and 2855 cm<sup>-1</sup> (aliphatic C–H stretching); (2) a decrease of the relative intensity at about 1541 cm<sup>-1</sup> (amide II); (3) a decrease of the relative intensity in the 1030–1080 cm<sup>-1</sup> region (polysaccharides or polysaccharide-like substances); (4) an increase of the relative intensity at about 1650 cm<sup>-1</sup> (aromatic C=C, C=O stretching of amide groups (amide I band), quinonic C=O and/or C=O of H-bonded conjugated ketones); (5) a marked intensity increase at about 1420 cm<sup>-1</sup> (carboxylic acids and amide III); (6) an increase of the relative intensity at about 1330 cm<sup>-1</sup> (aromatic primary and secondary amines); (7) a marked intensity increase at about 1126 cm<sup>-1</sup> (aliphatic OH). These changes tended to be more and more marked from V1-HAL to V5-HAL with the increasing proportion of cow dung in the mixtures.

Thus, these observations indicated the loss of aliphatic materials, N-containing groups (probably proteinaceous materials) and carbohydrates and the enrichment of carboxylic acids and aromatic structures in HAL fractions after vermicomposting. The FT-IR data further confirmed and complemented the previous data suggesting that the HAL fraction in vermicomposts had more aromaticity and polycondensation than that noted in the initial substrates.

### 3.2.4. Scanning electron microscopy (SEM)

The SEM micrographs of the HAL fractions isolated from the initial substrates and vermicomposts are presented in Fig. 4. The results reveal that the FSS-HAL has a loosely honeycomb structure characterized by a predominance of flakes and fragments adhering on the surface, while the FCD-HAL is characterized by a non-firm fragmentation and some cell-like materials embed on the surface.

After vermicomposting, the HAL fractions exhibited a distinct physical appearance, being close-grained and lumpy (see the microstructure in V5-HAL as most representative all of this appearance). This indicates that vermicomposting leads to a more compacted structure of HAL fractions than that noted in either of the initial FSS-HAL and FCD-HAL. Authors suggested that the condensation lumpy structures possessed superior property in the permeability of water and ventilation property than noted in FSS and FCD, which would avoid or reduce soil agglomeration in the soil application process, making the vermicomposts superb soil organic amendments. The results confirmed and complemented previous findings in elemental and functional group composition, and fluorescence and FT-IR spectra.

## 4. Conclusions

Vermicomposting of sewage sludge spiked with cow dung resulted in a decrease in pH value, TOC content and C/N ratio, and an increase in EC, TEC and HAC contents and HR and HI values. Additionally, TOC loss and humification degree in vermicomposts became more and more marked with the increasing proportion of cow dung in the substrates, indicating the addition of cow dung could improve chemical stability and organic matter humification of sewage sludge.

After vermicomposting, the HAL fractions extracted from the substrates were characterized by small aliphatic character and proteinaceous and polysaccharide-like components, high acidic functional group content and larger amounts of aromaticity, polycondensation and humification, approaching those typical of soil HA [44]. In particular, the features in vermicompost of cow dung alone were the most marked.

In sum, these results imply that vermicomposting is an efficient technology for transforming fresh organic matter in sewage sludge into humic substances, and thus improved quality of sewage sludge as a soil organic amendment. Additionally, mixture of sewage sludge and cow dung seemed to produce better vermicompost than



that of sewage sludge alone, in terms of HAL fraction quality of the final product.

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