



Fluorescence excitation–emission matrix spectroscopy with regional integration analysis for characterizing composition and transformation of dissolved organic matter in landfill leachates

Xiao-Song He^{a,b}, Bei-Dou Xi^{a,*}, Zi-Min Wei^c, Yong-Hai Jiang^a, Yu Yang^a, Da An^{a,b}, Jin-Ling Cao^a, Hong-Liang Liu^a

^a Laboratory of Water Environmental System Engineering, Chinese Research Academy of Environmental Science, Beijing 100012, China

^b School of Environment, Beijing Normal University, Beijing 100875, China

^c Life Science College, Northeast Agricultural University, Harbin 150030, China

ARTICLE INFO

Article history:

Received 4 January 2011

Received in revised form 2 March 2011

Accepted 12 March 2011

Available online 22 March 2011

Keywords:

Excitation–emission matrix (EEM) spectroscopy

Fluorescence regional integration (FRI)

Dissolved organic matter (DOM)

Landfill leachates

ABSTRACT

Dissolved organic matter (DOM) obtained from landfill leachates was separated into hydrophobic base, hydrophilic matter (HIM), hydrophobic acid (HOA), and hydrophobic neutral fractions. The composition and transformation of the DOM and its fractions were investigated. The results show that the DOM isolated from young, intermediate, and old landfill leachates were mainly composed of tyrosine-, tryptophan-, and humic- and fulvic-like substances, respectively. The primary fractions of the DOM in leachates were HOA and HIM. The HOA and HIM fractions from young leachates predominantly contained tryptophan- and tyrosine-like materials, respectively. The HOA fractions in intermediate and old leachates were mainly composed of humic- and fulvic-like materials, whereas the HIM fractions were dominated by tryptophan-like materials and humic- and fulvic-like substances. The hydrophobic organic fractions and humic- and fulvic-like substances increased with time, whereas the HIM and the tyrosine-like materials decreased during the landfill process, rendering biological processing of leachates ineffective.

© 2011 Elsevier B.V. All rights reserved.

1. Introduction

Landfill leachates are composed of a large number of organic substances released from the microbial degradation of municipal solid waste (MSW). These require sufficient treatment before they are discharged into the environment [1]. Dissolved organic matter (DOM) accounts for more than 85% of total organic matter in terms of organic carbon in leachates and is the main component of leachate treatment [2]. In addition, DOM can interact with several inorganic and organic pollutants due to the presence of oxygen-containing and aromatic functional groups in its constituents, which play a significant biochemical and geochemical role in landfill systems [3]. Studies on the compositions of DOM in landfill leachates can guide treatment process selection and establish the groundwork for environmental risk assessment of leachates [4]. As leachate compositions vary significantly with landfill age and may pose additional challenges for leachate treatment techniques

[5], investigating changes in leachate DOM during the landfill process is imperative.

DOM in leachates is composed of a heterogeneous mixture of degradation products. Fractionation can reduce DOM complexity, and a number of studies have applied the DOM fractionation method based on compound hydrophobicity [2,6,7]. This method provides relevant data about DOM. Fluorescence excitation–emission matrix (EEM) spectroscopy has been widely used to investigate the composition and biogeochemical cycling of DOM due to its simplicity and sensitivity [8]. Several studies have characterized leachate DOM through EEM spectral analysis: Baker and Curry [9] investigated the EEM spectral characteristics of leachates from three contrasting landfill sites, Huo et al. [10] used EEM spectra to investigate the composition and transformation of the DOM in leachates of different ages, and Lu et al. [11] identified several fluorophores with multiple EEM peaks in leachate DOM by combining size–exclusion chromatography with EEM spectroscopy and parallel factor analysis. However, the analysis of EEM spectra in these previous studies was generally limited to visual identification of peaks or ratios of peak intensities, which cannot ascertain the heterogeneity of DOM samples. Fluorescence regional integration (FRI), a quantitative technique that integrates volumes beneath different excitation–emission regions in EEM spectra, can be used to

* Corresponding author at: No. 8, Dayangfang, Beiyuan Road, Beijing, China.

Tel.: +86 015810441270; fax: +86 10 84913133.

E-mail address: xibeidou@263.net (B.-D. Xi).

quantitatively analyze EEM spectra and to determine the configuration and heterogeneity of DOM [12–14]. However, data from EEM spectroscopy and FRI analysis used in combination to investigate the composition and transformation of DOM in landfill leachates are limited.

The objectives of the present study were to investigate the structural characteristics of DOM extracted from landfill leachates of different ages and to evaluate the transformation of leachate DOM compositions during the landfill process using EEM spectroscopy in combination with FRI analysis.

2. Materials and methods

2.1. Sample collection and pretreatment

Three leachate samples (L1, L2, and L3) were obtained from different units at the Asuwei Sanitary Landfill in Beijing, China. The landfill site has been operating since 1994, mainly for MSW. The average processing load of the landfill is approximately 2000 t d⁻¹, and the leachate quantity measures approximately 1000 m³ d⁻¹. Three samples were obtained by pumping from vertical sampling wells. Approximately 2 L of leachates was collected for each sample. Leachate sample L1 was obtained from cells younger than 3 years, L2 was collected from cells ranging in age from 3 to 10 years, and L3 was collected from cells that were more than 10 years old. All three leachate samples were filtered using 0.45 μm Whatman glass fiber filters. The filtrates were stored at 4 °C before analysis or further treatments.

2.2. Fractionation and collection of DOM fractions

DOM from the leachate samples was separated into hydrophobic base (HOB), hydrophilic matter (HIM), hydrophobic acid (HOA), and hydrophobic neutral (HON) fractions based on compound hydrophobicity using a modified procedure originated by Leenheer [15]. The modified procedure was as follows: First, the leachate filtrate was passed through an XAD-8 macroporous resin column (Rohm & Haas Corp., Philadelphia, PA). The effluent from the resin column was then collected and acidified to pH 2. The column was back-flushed with 0.1 mol L⁻¹ HCl (the resulting effluent was the HOB fraction). Next, the previously acidified effluent was passed through the XAD-8 resin column again (the effluent was the HIM fraction). The column was then back-flushed with 0.1 mol L⁻¹ NaOH (the effluent was the HOA fraction). Finally, the XAD-8 resin was Soxhlet-extracted with anhydrous methanol (the organic matter obtained was the HON fraction) [2,7].

2.3. Fluorescence spectroscopy

Before fluorescence analysis was performed, the dissolved organic carbon (DOC) of all samples was measured using an Analytik Jena Multi N/C 2100 TOC analyzer (Analytik Jena, Jena, Germany). All samples were diluted with 0.1 mol L⁻¹ phosphate buffer (pH 7). The final DOC content was set to approximately 8 mg L⁻¹. Fluorescence EEM spectroscopy was performed on each sample using a Perkin Elmer LS50B fluorescence spectrophotometer (Perkin Elmer, New Jersey, USA) at room temperature (~25 °C). Excitation and emission were simultaneously scanned at wavelengths ranging from 200 to 440 nm and from 300 to 500 nm, respectively, at 5 nm intervals. The slit widths were set at 10 nm for both excitation and emission monochromators, and the scan speed was set at 1200 nm min⁻¹.

Table 1

Composition of landfill leachates of different ages.

	Sample L1	Sample L2	Sample L3
HOA	51.90%	55.86%	61.59%
HON	3.81%	5.86%	5.80%
HOB	2.77%	0.90%	1.45%
HIM	41.52%	37.39%	31.16%

2.4. Data processing

After the scatter in the areas affected by first- and second-order Rayleigh and Raman scattering was regulated using interpolation [16], the FRI technique was adopted for EEM spectral data analysis [12]. Hierarchical cluster analysis (HCA) of the data obtained from the FRI analysis was performed using SPSS 16.0 software (SPSS International, Chicago, USA) and with Ward's method, which uses the squared Euclidean distance as a similarity measure. Finally, Origin 8.0 software (OriginLab, Los Angeles, USA) was used for figure processing.

3. Results and discussion

3.1. Fraction distributions of DOM in leachates

DOM obtained from the landfill leachates was fractionated into four classes according to the hydrophobic/hydrophilic character of the molecules. The fractionation results of the three samples are shown in Table 1. Hydrophobic organic fractions (including HOA, HOB, and HON fractions), which collectively accounted for more than 58.48% of the DOM in terms of DOC, were dominant; these hydrophobic carbon fractions increased with increasing landfill age (from 58.48% to 68.84%). The HOA fraction was the most abundant fraction (>51.90%) and increased with time (from 51.90% to 61.59%), indicating that it constituted much of the organic substance composition in the wastewater. The HOB fraction was the least abundant, constituting less than 2.77% of the DOC, consistent with the findings reported by Zhang et al. [2]. The proportions of HON fractions in all DOM samples were similar (3.81–5.86%), and they were in the intermediate range among the three hydrophobic fractions. The hydrophilic fraction (HIM) constituted more than 31.16% of the DOC in the leachates and had the tendency to decrease as landfill age increased (from 41.52% to 31.16%), indicating that the fraction might have disintegrated or converted into hydrophobic components as landfill age increased. Compared with HIM, hydrophobic materials are not easily used for the energy requirements of microorganisms [3]. Therefore, the increase in hydrophobic matter and the decrease in hydrophilic materials in the leachates stabilize the DOM, rendering the biological process for leachates ineffective.

3.2. Structural characteristics of DOM in landfill leachates

3.2.1. DOM and its fractions in young landfill leachates

The EEM spectra of the DOM and its corresponding fractions from different source ages are shown in Fig. 1. Four protein-like peaks (B1, B2, T1, and T2) were detected in the non-fractionated DOM extracted from the young leachate source (Fig. 1a). Previous studies [9,17,18] have shown that peaks B1 and B2 are associated with tyrosine-like materials as free molecules or bound to amino acids and proteins, whereas peaks T1 and T2 are related to tryptophan-like compounds present as free molecules or bound in proteins, peptides, or humic structures. In addition to protein-like peaks, a humic acid-like peak (peak C) was also observed in the sample; peak C was partly covered by the nearby peak (T1), and its fluorescent center was poorly understood. Previous research

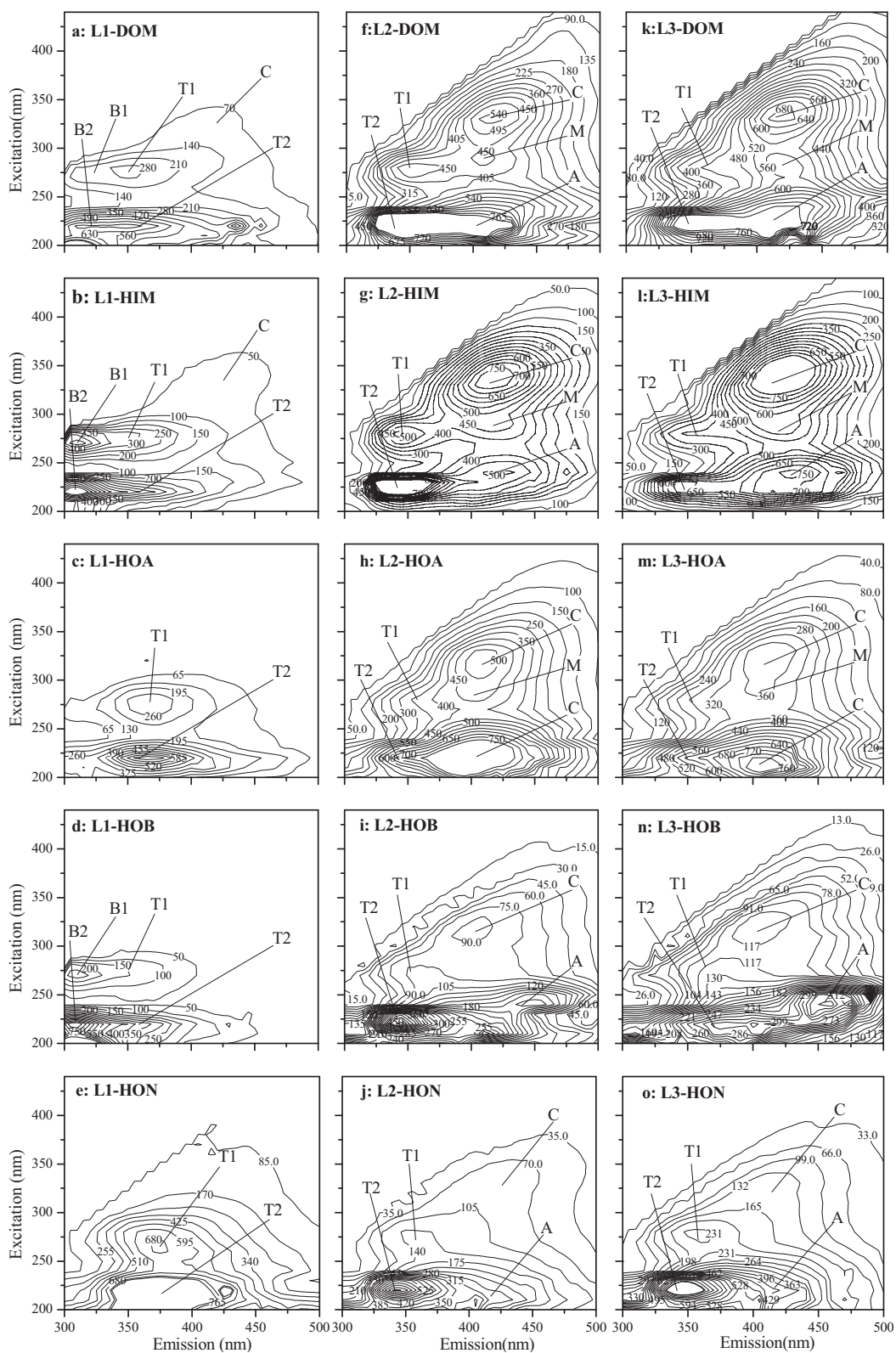


Fig. 1. EEM spectra of DOM and its corresponding fractions isolated from landfill leachates of different ages.

using DOM analysis has suggested that peak C is attributable to humic-like organic substances; different molecular components derived from lignin and other degraded plant materials in the MSW are potential contributors to this peak [13]. The isolated sample most similar to the non-fractionated DOM is the HIM fraction (Fig. 1b). However, compared with the non-fractionated DOM,

the fluorescence region for peak C was more evident in the HIM fraction. Similar to young leachate DOM and its HIM fraction, the HOB fraction isolated from sample L1 exhibited four protein-like peaks (Fig. 1d); however, the EEM spectral configuration of the HOB fraction was slightly different from the configurations of the above-mentioned samples in that the humic acid-like fluorescence region

(peak C) was much less evident in the HOB fraction. In contrast to the abovementioned samples, T1 and T2 were the only peaks in the HOA and HON fractions (Fig. 1c and e), indicating that tryptophan-like substances were the major components in these fractions.

FRI can reveal the configuration and heterogeneity of DOM. It has been widely used to quantitatively analyze all wavelength-dependent fluorescence intensity data from EEM spectra. In the present study, the EEM was divided into six regions using consistent excitation and emission wavelength boundaries based on previous research [12] (Fig. 2). Peaks at shorter excitation wavelengths (<250 nm) and shorter emission wavelengths (<380 nm) are associated with tyrosine- and tryptophan-like compounds (Regions I and II) [19]. On the other hand, peaks at shorter excitation wavelengths (<250 nm) and longer emission wavelengths (>380 nm) are related to fulvic acid-like substances (Region III) [20]. Peaks located at longer excitation wavelengths (>250 nm) and shorter emission wavelengths (<380 nm) are also correlated with tyrosine- and tryptophan-like materials (Regions IV and V) [17,20]. In addition, fluorescence emitted by soluble microbial byproduct-like materials is commonly seen in Region V [12]. Peaks at longer excitation wavelengths (>250 nm) and longer emission wavelengths (>380 nm) represent humic acid-like organic fractions (Region VI) [21] (Fig. 2). The volumetric integration under the EEM within each region, normalized to the projected excitation–emission area within that region and to the DOC concentration, resulted in a normalized region-specific EEM volume ($P_{i,n}$) [12].

The distributions of $P_{i,n}$ for the six EEM regions in the DOM and its corresponding fractions isolated from leachates of different ages are presented in Table 2. For the non-fractionated DOM and the HIM and HOB fractions extracted from L1, the highest and lowest percentages of $P_{i,n}$ occurred in Region I and Region VI, respectively, indicating that tyrosine-like materials were the major component in the three samples. In contrast to the other samples extracted from L1, the highest percentage of the $P_{i,n}$ in the HOA and HON fractions occurred in Region II, indicating that tryptophan-like substances were the major component in the two fractions. This quantitative result is consistent with the location of the EEM peaks within Region II of the delineated regions (Fig. 1c and e). Similar to the non-fractionated DOM and the HIM and HOB fractions from L1, the percentage of $P_{i,n}$ for Region VI was lowest in the HOA fraction, indicating that a small concentration of humic-like substances was present in the DOM extracted from the L1 sample. However, for the HON fraction isolated from L1, the lowest percentage of $P_{i,n}$ appeared in Region IV, indicating that tyrosine-like materials were lowest in the fraction.

3.2.2. DOM and its fractions in intermediate and old landfill leachates

The EEM spectra of DOM and its corresponding fractions from samples L2 and L3 were similar (Fig. 1f and k). Two protein-like peaks (B1 and B2) disappeared but a fulvic acid-like peak and a humic acid-like peak (A and M) appeared in the two samples. In addition, peak C was designated as a primary peak in the DOM extracted from L2 and L3. According to Marhuenda-Egea et al. [13] and He et al. [14], peak A is related to fulvic-like compounds and peak M is associated with humic-like materials. These results suggest that fulvic- and humic-like substances were the important compounds in L2 and L3 DOM. Similar to the corresponding DOM, the HIM fraction isolated from the two samples also exhibited five peaks (Fig. 1g and l), although the fluorescence intensities of the DOM and its corresponding HIM fraction were different. In contrast to the corresponding DOM samples, the protein-like peaks (T1 and T2) almost disappeared (Fig. 1h and m) but the humic acid- and fulvic acid-like peaks (C and A) persisted in the HOA fractions extracted from L2 and L3 DOM. These findings indicate that the two fractions consisted of high concentrations of humic- and fulvic-like

substances. Peaks T1, T2, C, and A were all observed in the HOB fractions isolated from L2 and L3 DOM (Fig. 1i and n), indicating that the two fractions consisted of protein-, humic-, and fulvic-like substances. In contrast to the corresponding HOB fractions, the tryptophan-like peaks became stronger and the fulvic acid-like peak almost disappeared in the HON fractions isolated from L2 and L3 (Fig. 1j and o). These results suggest that the major compounds in the HON fractions extracted from L2 and L3 were composed of tryptophan-like materials.

The results of the FRI analysis of the DOM and its fractions from L2 and L3 are shown in Table 2. For the non-fractionated DOM extracted from L2, the $P_{i,n}$ value for Regions II and V in combination (tryptophan-like substances) was 0.449, higher than the $P_{i,n}$ values for Regions I and IV (tyrosine-like materials) and Regions III and VI (humic- and fulvic-like materials). In contrast to the non-fractionated DOM extracted from L2, the $P_{i,n}$ value for Regions III and VI was highest in DOM extracted from L3, suggesting that slightly more humified (humic- or fulvic-like) materials were present in the sample. For the HIM fractions isolated from L2 and L3 DOM, the distribution of organic materials was similar to that in the corresponding non-fractionated DOM. The $P_{i,n}$ values for the humic- and fulvic-like regions (Regions III and VI) in the HOA fractions isolated from L2 and L3 DOM were 0.421 and 0.410, respectively, higher than those for the tryptophan-like regions (Regions II and V) and the tyrosine-like regions (Regions I and IV) in the corresponding fractions. These results suggest that humic- and fulvic-like substances were the major compounds in the HOA fractions extracted from L2 and L3. The percentages of $P_{i,n}$ for the tryptophan-like regions were highest in the HOB and HON fractions extracted from L2 DOM, indicating that the two fractions mainly consisted of tryptophan-like materials. Similarly, the major compounds in the HON fractions extracted from L3 DOM were tryptophan-like materials (Regions II and V). However, the humic- and fulvic-like substances (Regions III and VI) accounted for much of the organic matter in the HOB fraction extracted from L3 (Table 2).

3.3. Transformation of DOM during the landfill process

The $P_{i,n}$ values of the six EEM regions for the DOM and its corresponding fractions in leachates of different ages are shown in Fig. 3. The $P_{i,n}$ values for Regions I and IV in the non-fractionated DOM dramatically decreased as landfill processing time progressed, whereas those for Regions III and VI significantly increased during the process, indicating that the landfill process of MSW is characterized by a decrease in tyrosine-like materials and an increase in humic- and fulvic-like substances. These results suggest that the humification degree of organic matter in the leachates increased as landfill time progressed. Hudson et al. [18] reported that tyrosine-like materials are easily biodegraded compared with humic- and fulvic-like substances. The decrease in tyrosine-like materials and the increase in humic and fulvic-like substances during the landfill of MSW hence stabilize organic matter. The $P_{i,n}$ for Regions II and V (tryptophan-like materials) showed disorder during the process. Tryptophan-like compounds present as free molecules or bound in proteins and peptides are easily used for the energy requirements of microorganisms, whereas those bound in humic structures resist biodegradation. The variation in the distribution of the tryptophan-like compounds of different configurations may cause the disarray in $P_{i,n}$ for Regions II and V as landfill age increased. The conversion of the HIM fractions was similar to that of the non-fractionated DOM. For the HOA fractions, the $P_{i,n}$ for Regions II and V (tryptophan-like materials) decreased with time, whereas the $P_{i,n}$ values for the other regions were perturbed. For the HOB fractions, the $P_{i,n}$ for the humic- and fulvic-like substances (Regions III and VI) increased as landfill time progressed and that

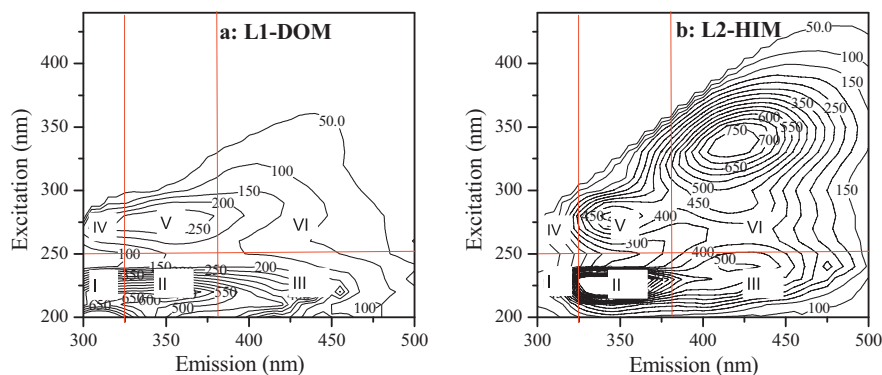


Fig. 2. The six EEM regions obtained using consistent excitation and emission wavelength boundaries.

Table 2

The distribution of $P_{i,n}$ in the six EEM regions for DOM and its corresponding fractions isolated from landfill leachates of different ages.

Sample	Region I	Region II	Region III	Region IV	Region V	Region VI	P_h/P_p^a
L1-DOM	0.312	0.280	0.138	0.127	0.104	0.040	0.216
L1-HIM	0.320	0.183	0.070	0.266	0.122	0.039	0.123
L1-HOA	0.205	0.347	0.164	0.089	0.148	0.047	0.267
L1-HOB	0.468	0.233	0.050	0.175	0.066	0.009	0.062
L1-HON	0.137	0.338	0.230	0.065	0.161	0.070	0.429
L2-DOM	0.136	0.305	0.223	0.064	0.144	0.128	0.542
L2-HIM	0.088	0.265	0.167	0.082	0.200	0.197	0.572
L2-HOA	0.120	0.278	0.296	0.047	0.135	0.125	0.727
L2-HOB	0.193	0.361	0.231	0.038	0.100	0.078	0.446
L2-HON	0.209	0.383	0.204	0.043	0.101	0.061	0.359
L3-DOM	0.105	0.266	0.261	0.054	0.144	0.169	0.753
L3-HIM	0.078	0.205	0.251	0.064	0.166	0.236	0.949
L3-HOA	0.135	0.274	0.286	0.050	0.131	0.124	0.694
L3-HOB	0.165	0.263	0.312	0.051	0.109	0.100	0.702
L3-HON	0.205	0.372	0.199	0.053	0.111	0.060	0.350

$$^a P_h/P_p = \sum_{III+VI} / \sum_{I+II+IV+V}$$

for the tyrosine-like substances (Regions I and IV) decreased during the process. In contrast to the other samples, the humic- and fulvic-like substances (Regions III and VI) in the HON fractions decreased as landfill age increased, whereas the change in the concentrations of tryptophan-like (Regions II and V) and tyrosine-like (Regions I and IV) materials in the HON fractions resulted in disorder during the landfill process. Compared with the HON fractions isolated from L2 and L3 DOM, the maximum fluorescence center of peaks T1 and T2 in the L1 HON leachates slightly shifted toward longer wave-

lengths (Fig. 1e), which caused the $P_{i,n}$ values for Regions II and VI to increase.

Huo et al. [10] investigated the composition and transformation of DOM in leachates using fluorescence peak location analysis. However, the identification of fluorescence peak centers is at times difficult [9,10], rendering analysis inapplicable. The composition and transformation of DOM can be easily assessed by EEM spectroscopy with FRI analysis, making their combination a valuable research tool for investigating organic matter transformation.

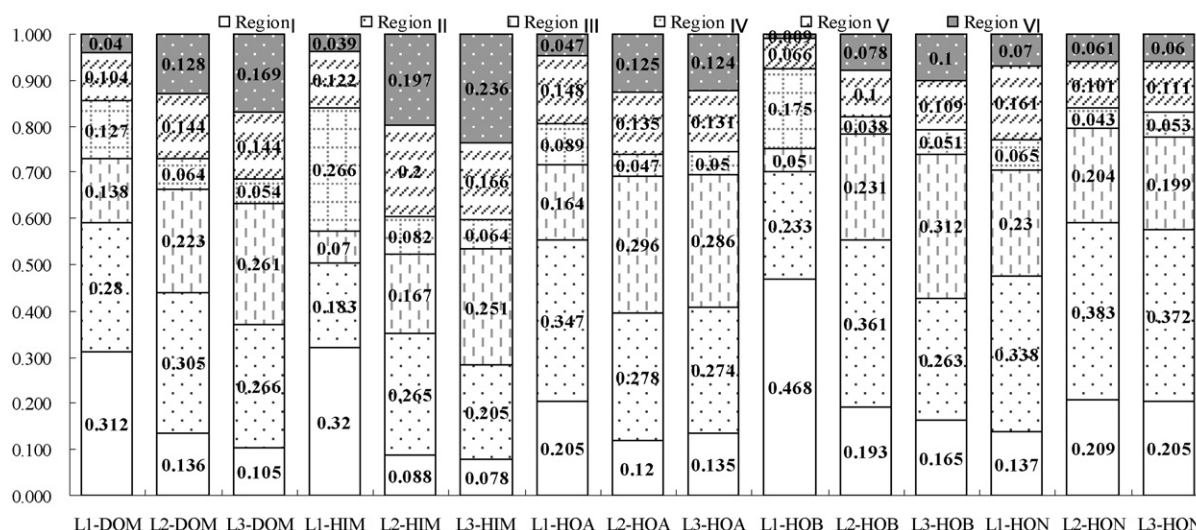


Fig. 3. The change of $P_{i,n}$ values for the DOM and its corresponding fractions during the landfill process.

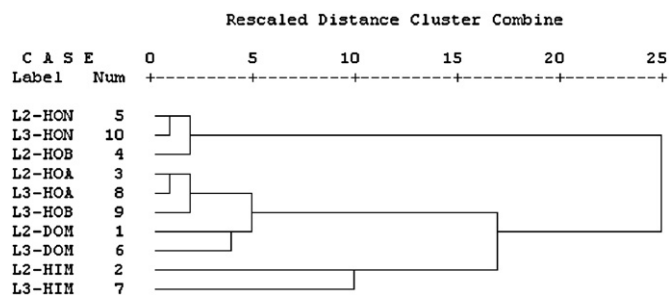


Fig. 4. HCA analysis among different samples based on the data from the FRI analysis.

The DOM and its fractions extracted from L1 were clearly different from those extracted from L2 and L3. However, the EEM spectra of DOM and its corresponding fractions from L2 and L3 were similar. HCA was used to analyze the difference between DOM and its fractions extracted from L2 and L3 to better understand the transformation characteristics of organic matter during the intermediate and old landfill periods (Fig. 4). HCA is an unsupervised pattern detection method that partitions all cases into smaller groups or clusters of relatively similar cases. According to Zbytniewski and Buszewski [22], the smaller distance between the samples indicates higher similarity between them. For the samples extracted from L2 and L3, the rescaled distance between the non-fractionated DOM samples was larger than the rescaled distances between the HOA fractions and between the HON fractions. However, the rescaled distance between the non-fractionated DOM samples was smaller than the rescaled distances between the HIM fractions and between the HOB fractions. These results indicate that non-fractionated DOM significantly changed during the landfill process. These changes occurred mainly in the HIM and HOB fractions; the HOA and HON fractions minimally changed during the process.

The HIM fractions were composed of tryptophan-, humic-, and fulvic-like substances. The tryptophan-like materials were easily used by the microorganisms as described above. Humic- and fulvic-like substances present in the HIM fractions were hydrophilic, and their molecular weight (MW) values were low relative to those of the HOA fractions [7], which facilitated the microbial degradation of the humic- and fulvic-like materials. The HOB fractions consisted mostly of tryptophan-like materials, and their apparent MW values ranged from 100 to 200 Da [11]. The HOB fractions could be easily removed by landfill treatments. This result is consistent with the findings reported by Lu et al. [11]. The HOA fractions exhibited three EEM peaks resembling those of humic-like substances. According to previous studies [7,11], HOA fractions are composed of the pyrenyl functional group and their MW values are the highest among all fractions, which both increase their residence time in landfills. The HON fractions also consisted of the tryptophan-like compounds. However, Lu et al. [11] suggested that HON fractions are precursors of HOA fractions during biosynthesis and that their MW values are within ranges similar to those in HOA fractions. The HON fractions in the present study were thus not easily degraded by the microorganisms.

3.4. Selection of leachate treatment process during the landfill process

Leachate composition is complex and varies with time. As prediction of effective treatment techniques for leachates is complicated, parameters that characterize leachates and calculate their treatment efficiency have been developed. The ratio of biochemical oxygen demand for 5 days (BOD_5) to chemical oxygen demand is a

common parameter [23], but the BOD_5 test is time consuming and delays analysis of potential pollution events.

Peaks B1, B2, T1, and T2 are related to bacterial activity and represent the presence of a labile organic substrate or the product of microbial activity. Previous studies have demonstrated that the fluorescence intensity in protein-like regions is correlated with the concentration of organic materials, which have a high potential for biodegradation [9,20,24]. On the contrary, peaks C, A, and M are associated with humic- and fulvic-like materials, which are not easily used by microorganisms [18]. Huo et al. [25] showed that protein-like organic matter is readily removed by biological processing and humic- and fulvic-like matter can be removed by reverse osmosis. The ratio of $P_{i,n}$ for the humic- and fulvic-like regions (Regions III and VI) to $P_{i,n}$ for the protein-like regions (Regions I, II, V, and IV) (P_h/P_p) may hence be used to predict a treatment technique for wastewater. More appropriate biological treatments require lower P_h/P_p ratios. The P_h/P_p ratio of the DOM extracted from L1 was 0.216, whereas the P_h/P_p ratios of the DOM extracted from L2 and L3 were 0.542 and 0.753, respectively, indicating an increasing trend during the landfill process. These results demonstrate that biological treatment is effective for young landfill leachates and that physicochemical processes are more appropriate for the treatment of stabilized leachates because of the high fraction of humic- and fulvic-like organic materials.

4. Conclusions

The results obtained from this work led to the following conclusions:

- (1) The content of hydrophobic organic fractions was higher than that of HIM in leachate DOM. The landfill process was characterized by an increase in HOA and a decrease in HIM.
- (2) DOM extracted from the young and intermediate landfill leachates were mainly composed of tyrosine- and tryptophan-like substances, respectively, whereas DOM extracted from old landfill leachates consisted of mostly humic- and fulvic-like matter. The humification degree of the DOM increased with increasing landfill age.
- (3) The primary fractions in leachate DOM were HOA and HIM. The HOA fraction isolated from young landfill leachates contained mostly tryptophan-like materials, whereas the HIM fraction predominantly consisted of tyrosine-like matter. For intermediate and old landfill leachates, the HOA fractions were mainly composed of humic- and fulvic-like materials. Tryptophan-like substances were the dominant structures in the HIM isolated from intermediate leachate DOM, whereas humic- and fulvic-like materials dominated in the HIM isolated from old leachate DOM.
- (4) The increase in $P_{i,n}$ for the humic- and fulvic-like regions and the decrease in $P_{i,n}$ for the protein-like regions render biological processing of leachates during the landfill process less ineffective.

Acknowledgements

This work was financially supported by the National Natural Science Foundation of China (Grant Nos. 50878201 and 50908219), the Heilongjiang Provincial Natural Science Foundation of China (No. C200917) and the National Public Benefit (Environmental) Research Foundation of China (No. 200909079).

References

- [1] P.J. He, J.F. Xue, L.M. Shao, G.J. Lia, D.J. Lee, Dissolved organic matter (DOM) in recycled leachate of bioreactor landfill, *Water Res.* 40 (7) (2006) 1465–1473.

- [2] L. Zhang, A.M. Li, Y.F. Lu, L. Yan, S. Zhong, C.L. Deng, Characterization and removal of dissolved organic matter (DOM) from landfill leachate rejected by nanofiltration, *Waste Manage.* 29 (3) (2009) 1035–1040.
- [3] A.I. Pelaez, J. Sanchez, G. Almendros, Bioreactor treatment of municipal solid waste landfill leachates: characterization of organic fractions, *Waste Manage.* 29 (1) (2009) 70–77.
- [4] J.A. Leenheer, J.P. Croue, Characterizing aquatic dissolved organic matter, *Environ. Sci. Technol.* 37 (1) (2003) 18–26.
- [5] Z.Y. Lou, Y.C. Zhao, T. Yuan, Y. Song, H.L. Chen, N.W. Zhu, R.H. Huan, Natural attenuation and characterization of contaminants composition in landfill leachate under different disposing ages, *Sci. Total Environ.* 407 (10) (2009) 3385–3391.
- [6] X. Xia, Z. Yang, X. Zhang, Effect of suspended-sediment concentration on nitrification in river water: importance of suspended sediment–water interface, *Environ. Sci. Technol.* 43 (10) (2009) 3681–3687.
- [7] D.J. Seo, Y.J. Kim, S.Y. Ham, D.H. Lee, Characterization of dissolved organic matter in leachate discharged from final disposal sites which contained municipal solid waste incineration residues, *J. Hazard. Mater.* 148 (3) (2007) 679–692.
- [8] F.C. Wu, Y.R. Cai, D. Evans, P. Dillon, Complexation between Hg(II) and dissolved organic matter in stream waters: an application of fluorescence spectroscopy, *Biogeochemistry* 71 (3) (2004) 339–351.
- [9] A. Baker, M. Curry, Fluorescence of leachates from three contrasting landfills, *Water Res.* 38 (10) (2004) 2605–2613.
- [10] S.L. Huo, B.D. Xi, H.C. Yu, L.S. He, S.L. Fan, H.L. Liu, Characteristics of dissolved organic matter (DOM) in leachate with different landfill ages, *J. Environ. Sci.* 20 (4) (2008) 492–498.
- [11] F. Lu, C.H. Chang, D.J. Lee, P.J. He, L.M. Shao, A. Su, Dissolved organic matter with multi-peak fluorophores in landfill leachate, *Chemosphere* 74 (4) (2009) 575–582.
- [12] W. Chen, P. Westerhoff, J.A. Leenheer, K. Booksh, Fluorescence excitation–emission matrix regional integration to quantify spectra for dissolved organic matter, *Environ. Sci. Technol.* 37 (24) (2003) 5701–5710.
- [13] F.C. Marhuenda-Egea, E. Martinez-Sabater, J. Jorda, R. Moral, M.A. Bustamante, C. Paredes, M.D. Perez-Murcia, Dissolved organic matter fractions formed during composting of winery and distillery residues: evaluation of the process by fluorescence excitation–emission matrix, *Chemosphere* 68 (2) (2007) 301–309.
- [14] X. He, B. Xi, Z. Wei, X. Guo, M. Li, D. An, H. Liu, Spectroscopic characterization of water extractable organic matter during composting of municipal solid waste, *Chemosphere* 82 (4) (2011) 541–548.
- [15] J.A. Leenheer, Comprehensive approach to preparative isolation and fractionation of dissolved organic carbon from natural waters and wastewaters, *Environ. Sci. Technol.* 15 (5) (1981) 578–587.
- [16] M. Bahram, R. Bro, C. Stedmon, A. Afkhami, Handling of Rayleigh and Raman scatter for PARAFAC modeling of fluorescence data using interpolation, *J. Chemometr.* 20 (3–4) (2006) 99–105.
- [17] P.G. Coble, Characterization of marine and terrestrial DOM in seawater using excitation–emission matrix spectroscopy, *Mar. Chem.* 51 (4) (1996) 325–346.
- [18] N. Hudson, A. Baker, D. Wardb, D.M. Reynolds, C. Brunsdond, C.M.C. Cynthia, S. Browning, Can fluorescence spectrometry be used as a surrogate for the biochemical oxygen demand (BOD) test in water quality assessment? An example from South West England, *Sci. Total Environ.* 391 (1) (2008) 149–158.
- [19] S.R. Ahmad, D.M. Reynolds, Monitoring of water quality using fluorescence technique: prospect of on-line process control, *Water Res.* 33 (9) (1999) 2069–2074.
- [20] S. Mounier, R. Braucher, J.Y. Benaim, Differentiation of organic matter's properties of the Rio Negro basin by crossflow ultra-filtration and UV-spectrofluorescence, *Water Res.* 33 (10) (1999) 2363–2373.
- [21] R. Artinger, C. Buckau, S. Geyer, P. Fritz, M. Wolf, J.I. Kim, Characterization of groundwater humic substances: influence of sedimentary organic carbon, *Appl. Geochem.* 15 (1) (2000) 97–116.
- [22] R. Zbytniewski, B. Buszewski, Characterization of natural organic matter (NOM) derived from sewage sludge compost. Part 2: multivariate techniques in the study of compost maturation, *Bioresour. Technol.* 96 (4) (2005) 479–484.
- [23] E.H.C. Sarah, H.B. Treavor, C.G. Katherine, G.T. Timothy, Effect of landfill characteristics on leachate organic matter properties and coagulation treatability, *Chemosphere* 81 (7) (2010) 976–983.
- [24] A. Baker, R. Inverarity, Protein-like fluorescence intensity as a possible tool for determining river water quality, *Hydrol. Process* 18 (15) (2004) 2927–2945.
- [25] S.L. Huo, B.D. Xi, H.C. Yu, H.L. Liu, Dissolved organic matter in leachate from different treatment processes, *Water Environ. J.* 23 (1) (2009) 15–22.