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Effect of inoculating microbes in municipal solid waste composting on characteristics of humic acid

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Abstract

Municipal solid waste (MSW) compost contains a significant amount of humic substances. In this study, the compost consisted of residual MSW with the metal, plastic and glass removed. In order to enhance degradation processes and the degree of composting humification, complex microorganisms (*Bacillus casei*, *Lactobacillus buchneri* and *Candida rugopelliculosa*) and ligno-cellulolytic (*Trichoderma* and White-rot fungi) microorganisms were respectively inoculated in the composting process. During the MSW composting, humic acid (HA) was extracted and purified. Elements (C, N, H, O) and spectroscopic characteristics of the HA were determined using elementary analyzer, UV, Fourier transform infrared (FTIR), and fluorescence spectroscopy. The elements analysis, UV, FTIR and fluorescence spectra all led to the same conclusion, that is inoculations with microbes led to a greater degree of aromatization of HA than in the control process (CK) with no inoculation microbes. This indicated that inoculation with microbes in composting would improve the degree humification and maturation processes, in the following order: lingo-cellulolytic > complex microorganisms > CK. And mixed inoculation of MSW with complex microorganisms and lingo-cellulolytic during composting gave a greater degree of HA aromatization than inoculation with complex microorganisms or lingo-cellulolytic alone. But comparing with the HA of soil, the HA of MSW compost revealed a lower degree of aromatization.

Keywords: Municipal solid wastes; Composting, Humic acid; Inoculation microbes; Spectra characteristics

1. Introduction

Composting is a well-known system for rapid stabilization and humification of organic matter (Adani et al., 1995), as well as an environmentally friendly and economical alternative method for treating solid organic waste (Huang et al., 2006). At present, many artificial measures have been developed, but improving composting efficiency is still a key issue. In China, with an increasing population, developing industry and economy, the output of municipal solid waste (MSW) has been growing. In recent years, the technology of MSW microbial composting has been gradually accepted in China since the organic matter content of the MSW exceeds 60% and the water content is about 50%, such that, when metal, plastic and glass are removed, the residual organic matter is suitable for composting (Wu et al., 2006). Therefore, the understanding of organic matter transformation throughout the composting process and proper evaluation of compost stability and maturity are essential for successful utilization of composts in the country.

During composting, readily degradable organic matter is used by microorganisms as a source of C and N. The end product (compost) consists of transformed, slowly-degradable compounds, intermediate breakdown products

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and the cell walls of dead microorganisms, which are classified together as humic substances (HSs). The importance of humic acid (HA) to soil ecology, fertility and structure, as well as its beneficial effects on plant growth, has resulted in an increase in the use of compost as an amendment to soils or as a substitute substrate for peat in container media (Chen et al., 1992; Avimelech et al., 1993). In addition, the HA characteristic of composting affects the compost maturity. At present, evaluating the maturity of the compost is still an open question (Tomati et al., 2000). Numerous biological, microbiological and physico-chemical techniques have been developed to characterize the agrochemical properties and the maturity of compost. Recently, compost maturity has been investigated via humification characteristics (Lguirati et al., 2005; Huang et al., 2006). Tomati et al. (2000) suggested monitoring the molecular weight of the HSs as an index of stability. Because the raw material represents a wide spectrum of organic wastes such as MSWs, sewage sludge, yard and green waste, animal manure and others types of waste (Chefetz et al., 1998), spectroscopic techniques such as Fourier transform infrared (FTIR), fluorescence spectroscopy and NMR have been used as characterization tools for studying the transformation of organic matter and along with evaluating the maturity of the compost (Garcia et al., 1992; Jimenez and Garcia, 1992; Ciavatta et al., 1993a,b; Chefetz et al., 1996; Baddi et al., 2003). The previous studies indicated an increase in polycondensed structures and the presence of more stable organic matter in the mature compost, which may be good indicators of humification processes of MSW composting with inoculation.

The results on the inoculation of different composting processes can be found in the literature (Biey et al., 2000; Ichida et al., 2001; Baheri and Meysami, 2002; Xi et al., 2005; Barrena et al., 2006). It seems clear that inoculation can have a positive effect on composting, especially in the first thermophilic stage of the process (Tiquia et al., 1997; Bolta et al., 2003). But in most of the previous cited works only routine parameters of the composting process are profiled and compared between inoculated and non-inoculated treatments. Moreover, only an inoculum dosage is usually tested. There have been only very limited reports in regard to the spectroscopic characteristics of HA after inoculation with microbes during MSW composting. In addition, the composition of MSW from different sources could vary significantly, and the organic matter changes are not completely identical during the composting process.

In this study, inoculation microbes were used in MSW composting in order to increase the degree humification and improve the composting process. Investigations were conducted at the Daqing Meishang MSW Composing Plant in China. The characteristics of HA were studied using elementary analyzer, UV, FTIR as well as fluorescence spectroscopy during composting. The effect of the inoculation microbes on the composition characteristics of the HA in the compost was also determined. The results could provide a useful theoretical and practical foundation for industrialized MSW composting in China.

2. Materials and methods

2.1. Materials

The compost consisted of residual MSW, with the metal, plastic and glass removed. The nutrient element content was: C, 323.24 g kg^{-1}; N, 14.88 g kg^{-1}; P_2O_5, 10.02 g kg^{-1}; K_2O, 12 80 g kg^{-1}. The water content was 56%.

The complex microorganisms were composed of *Bacillus casei*, *Lactobacillus buchneri* and *Candida rugopelliculosa* provided by the Daqing Meishang Company (China). The ligno-cellulolytic microorganisms were composed of *Trichoderma* and White-rot fungi provided by the Zhongjia Biological Technique Company Limited (China). The microorganisms were, respectively, isolated from soil, sewage sludge, life waste, and pig manure, and stored in ultralow temperature freezer (MDF-792/AT). Before inoculated, the complex microorganisms were cultivated by malt extract agar (malt 15 g l^{-1}, agar 10 g l^{-1}), and the lingo-cellulolytic microorganisms were cultivated by potato dextrose agar (potato extract 200 g l^{-1}, agar 20 g l^{-1}). During cultivating process, the microbial colonies were counted using a standard dilution-plating procedure until to reach the desired concentration of 1×10^9 CFU ml^{-1} for composting inoculation. At the initial stage of composting, the microorganisms suspension was sprayed on the raw material. All the composting material was turned after inoculation to spread the microbes consortium.

2.2. Experimental methods

The experiment was conducted at the Daqing Meishang MSW compost plant from March 15 to May 18, 2003. The capacity of the plant is 200 tons per day. The waste collected from various sites of the city undergoes the following steps: (1) manual and mechanical sorting to remove metal, plastic and glass and (2) the residual MSW for composting.

The experimental design included four treatments and they were replicated three times. The treatments were: (1) control (CK) with no inoculation microbes; (2) treatment 1 (T1) with complex microorganisms (5 ml kg^{-1} dry MSW); (3) treatment 2 (T2) with ligno-cellulolytic microorganisms (5 ml kg^{-1} dry MSW); (4) treatment 3 (T3) with a mixed inoculation of complex microorganisms (2.5 ml kg^{-1} dry MSW) and ligno-cellulolytic microorganisms (2.5 ml kg^{-1} dry MSW). The inoculation microbes is all in the concentration of 1×10^9 CFU ml^{-1}. Each treatment consisted of windrows with a pyramidal of triangle (2.2 m × 4.5 m × 10 m, height × width × length). The composting process was implemented in two stages composting, the first stage decompounds the low-molecular weight compounds of composting, and the second stage curing composting. The main indices are shown in Table 1.

Table 1
Composting conditions

Condition	First stage composting	Second stage composting
Water content of input organic matter	60%	50%
Water content of output organic matter	30%	25–30%
Composting temperature	55 °C ⩽ T ⩽ 75 °C, lasted more than 5 days	
Composting period	28 days	35 days

Conditions: oxygen content ⩽10%; fermentation piles were mixed weekly; C/N was ⩽25:1.

2.3. Sampling

At the initial stage of the first stage and the final stage of the second stage of composting, a 500 g MSW sub-sample was taken from each windrow. From this, a 250 g fresh sample was analyzed for moisture content and water soluble matter. The rest was air dried and ground to pass through a 1 mm sieve. Organic C and HA were measured.

2.4. HA extraction

The isolation of humic material was performed according to the classic method of fractionation based on different solubility in water at different pH values (Stevenson, 1994). In the isolation process, a 200 g sample was shaken for 16 h at room temperature in a one litre mixed 1 l solution of 0.1 M NaOH and 0.1 M $Na_4P_2O_4$. The residue (humic and other insoluble compounds) was separated from the supernatant by centrifugation. Then, the supernatant was acidified (HCl; pH 1.0), such that the HA was purified and precipitated.

Elemental analysis (C, H, O, and N) was performed on freeze-dried samples of HA (0.5–1 mg) using an elementary analyzer (Elementar Vario EL, Germany).

The E_4/E_6 ratio, i.e. the ratio between absorbance at 465 and 665 nm was determined from the optical density of 2 mg samples of HA dissolved in 25 ml of 0.025 M $NaHCO_3$ (Chen et al., 1977).

2.5. Spectroscopic analysis

2.5.1. UV spectra

Samples of HA were dissolved in 0.05 M $NaHCO_3$. All samples were evaluated at a concentration of 16 mg l^{-1}. UV spectra of HA were recorded with an UV–Vis spectrophotometer Cary 50 (Varian, USA) in a 1 cm quartz cuvette, with scanning from 190 to 400 nm.

2.5.2. FTIR spectra

Dry HA samples (2 mg) were mixed with 200 mg of dry potassium bromide (KBr) and pressed into discs. The infrared spectra (400–4000 cm^{-1}) were recorded with the use of a Nicolet 550 Magna-IR spectrometer.

2.5.3. Fluorescence synchronous scan spectra

Fluorescence synchronous scanning was performed with a Perkin–Elmer Luminescence Spectrometer LS50B (USA). Samples of HA were dissolved in 0.05 M $NaHCO_3$. All samples were evaluated at a concentration of 20 mg l^{-1}. For synchronous scan fluorescence analysis, the slit width for emission and excitation wavelengths was 10 nm. Synchronous scan spectra were measured by scanning simultaneously over the range 300–600 nm with optimized wavelength difference, $\Delta\lambda = \lambda_{em} - \lambda_{ex} = 18$ nm (Tlombardi and Fjardim, 1999).

2.6. Microbiological assays

The densities of cultivable bacteria, actinomyceto, and fungi were estimated using a standard dilution-plating procedure, and replicated three times. Five grams of compost was suspended in 45 ml of serile water and shaken for 20 min. Ten-fold dilutions were made. Bacteria were quantified on yeast peptone glucose agar (yeast extract 5 g l^{-1}, peptone 5 g l^{-1}, glucose 10 g l^{-1}, agar 15 g l^{-1}) supplied with cycloheximide (100 mg l^{-1}), actinomyceto were quantified on Gause's synthetic agar (agar 18 g l^{-1}, starch 20 g l^{-1}) supplied with potassium dichromate (100 mg l^{-1}), and fungi were quantified in melting malt extract agar (malt 15 g l^{-1}, agar 10 g l^{-1}) supplied with antibiotics (citric acid 250 mg l^{-1}, chlortetracycline 50 mg l^{-1} and streptomycin 100 mg l^{-1}).

2.7. Statistical analysis

Elemental analysis (C, H, O, and N), E_4/E_6 and microorganisms concentration are presented as the average with standard error of triplicate samples. UV, FTIR and fluorescence spectra analyses were determined on a mixed sample from triplicate samples.

3. Results and discussion

3.1. Microbel community

The microbial community of different treatments was studied during MSW composting process (Fig. 1). The profile indicated that the microbial populations of inoculations were much larger than those of CK during the whole composting time, with the order as following: T3 > T2 > T1 > CK. In inoculation treatments, the microbial population of T1, T2, and T3 increased quickly in the initial stage, and later reached a peak level of 3.3×10^9, 7.6×10^9, 2.8×10^{10} CFU g^{-1} at seventh day. As a comparison, the corresponding peak level of microbes population in CK was only 4.3×10^8 CFU g^{-1} at fourteenth days. At the final stage of composting, the inoculated microorganisms decreased to a lower level due to depletion of substrates. This implied that inoculation was able to improve biodegradation efficiencies of the composting. In addition,

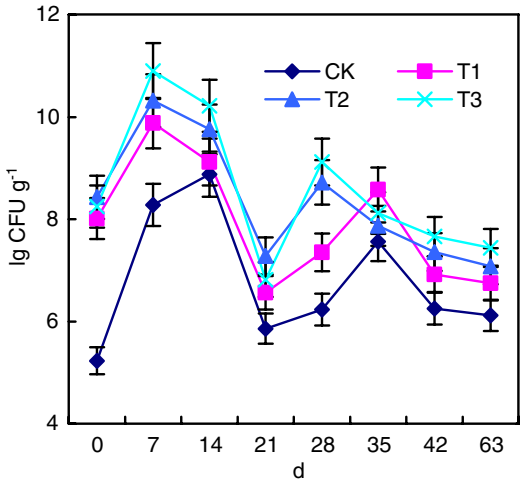

Fig. 1. Profile of microbial communities during composting process.

the final composting products contained rich microbial population compared to CK.

3.2. Elemental analysis and E_4/E_6 ratio

The elemental composition, the atomic ratios and the E_4/E_6 ratios of the HA extracted from MSW compost at initial and final stages are shown in Table 2. The levels of C and H decreased at the final stage of composting, while the levels of O increased and N remained constant. So, the C/N and O/C ratios decreased. The changes in these parameters occurred in all treatments at the final stage of composting, because degraded molecules, by the intense microbiological activity during composting, become reorganized to form more condensed compounds, richer in aromatic components. Furthermore, the degree of aromatization and humification of HA was increased at final stage of composting. Compared with the elemental composition and the atomic ratios of HA among all treatments, inoculation microbes would increase the degree of HA aromatization, in the following order: T3 > T2 > T1 > CK. But comparing HA from the MSW compost to reference soil HA (Table 2), shows that HA form MSW compost present lower levels of C and N, while the levels of O and H are higher. The MSW compost therefore presented HA with lower C/N and C/H ratios while the O/C and E_4/E_6 ratios were higher than the soil HA (Table 2). In other words, the HA from compost present a lower degree of aromatization than that from soil.

3.3. Spectroscopic analysis

3.3.1. UV spectroscopy

The UV spectra of HA exhibited an absorption band with the peak near 210 nm and a similar shoulder near 280 nm (Fig. 2). At the final stage of MSW composting, the UV absorption intensity in all treatments increased significantly. The absorption peaks of CK, T1, T2 and T3 shifted from 205 nm at the initial stage of composting to 208, 210, 210 and 212 nm, respectively. The absorption peak near 210 nm may be ascribed to an aromatic π–π electron transition. The reason for the red shift near 210 nm may be a reduction in $-NH_3$ and a high degree of polymerization that enhances the probability of bimolecular processes. In addition, the shoulder at 280 nm is important for evaluating the properties of HA, and is produced by an aromatic π–π^* transition or lignin. At the final stage of composting, the similar peak patterns at 280 nm were weakened in all treatments, suggesting that lignin decreased and the degree of aromatic polycondensation increased at the final stage of composting. At the final stage of composting, the HA absorption in the range

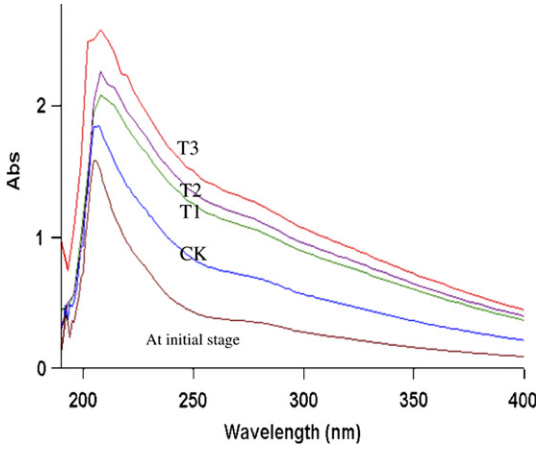

Fig. 2. HA UV spectra at the initial and final stages of MSW composting.

Table 2
Elemental composition, atomic ratios and E_4/E_6 of HA extracted from the MSW compost at initial and final stage

Treatments	C (%)	N (%)	H (%)	O (%)	E_4/E_6	Atomic ratios		
						C/N	C/H	O/C
At initial stage of composting	48.4 ± 2.21	2.8 ± 0.10	6.4 ± 0.21	38.4 ± 1.38	8.1 ± 0.32	20.1 ± 0.86	0.7 ± 0.03	0.7 ± 0.02
CK[a]	45.3 ± 1.53	2.7 ± 0.16	5.8 ± 0.24	42.1 ± 1.25	6.2 ± 0.28	16.2 ± 0.75	0.8 ± 0.03	0.6 ± 0.02
T1[a]	44.8 ± 1.15	2.7 ± 0.12	5.5 ± 0.20	43.3 ± 1.08	6.2 ± 0.32	15.4 ± 0.68	0.8 ± 0.04	0.6 ± 0.03
T2[a]	44.1 ± 0.88	2.7 ± 0.12	5.3 ± 0.32	44.0 ± 1.57	6.1 ± 0.25	14.6 ± 0.82	0.9 ± 0.02	0.6 ± 0.02
T3[a]	43.2 ± 1.06	2.8 ± 0.15	5.1 ± 0.35	44.8 ± 1.34	6.3 ± 0.20	14.1 ± 0.48	0.9 ± 0.04	0.6 ± 0.02
HA[b]	56.2	3.2	4.8	35.5	5.0	20.5	1.0	0.5

[a] CK, T1, T2 and T3 are all the treatments at final stage of composting.
[b] Soil HA values are taken form Schnizer (1978).

200–400 nm increased with microbial inoculation, in comparison with that of CK. The decomposition of the organic matter is reflected in the HA composition/humification in the compost at various stages. We monitored the UV spectral characteristics of HA at the initial and final stages of MSW composting in an attempt to understand the effect of inoculation microbes on HA transformation. In general, absorption in the UV–Vis spectra in the range of 200–700 nm gradually increased during composting. The rate of increase was higher in the shorter wavelength range than that in the longer wavelength range. The increase in UV absorption of the HA is probably due to an increase in π electrons, e.g. in unsaturated and/or aromatic compounds (Sugahara and Inokl, 1981). Therefore, at the final stage of composting (Fig. 2), the HA UV absorption spectra indicated that the inoculation microbes increased the humification process compared to samples with no inoculation microbes, in the order: T3 > T2 > T1 > CK.

3.3.2. HA FTIR spectroscopy

Unlike pure compounds, which have typically sharp absorption peaks, the HA from MSW composting has relatively fewer broad bands (Fig. 3); this may be a result of overlap of the absorptions of many similar function groups (MacCarthy et al., 1975). We also examined the characteristics of the typical absorption bands for HA in the FTIR spectra during MSW composting. The strong absorption band at 3400 cm^{-1} confirmed the presence of abundant OH and amino groups. Absorption peaks appeared near 2920 cm^{-1} (aliphatic C–H stretching), 1710 cm^{-1} (C=O stretching of COOH and ketonic C=O), 1600–1660 cm^{-1} (aromatic C=C and H bonded C=O), 1460 cm^{-1} (C–H bending of CH_2 or CH_3 groups) and 1400 cm^{-1} (stretching of COOH and deformation of phenol), 1233 cm^{-1} (C–O stretching of COOR, lignin). At the final stage of composting the absorption intensities at 2920, 2850, 1710 and 1540 cm^{-1} had clearly dropped. This indicated a decrease of low molecular weight carbohydrates (amylose, etc.) and an increase of aromatic condensation. It was also evi-

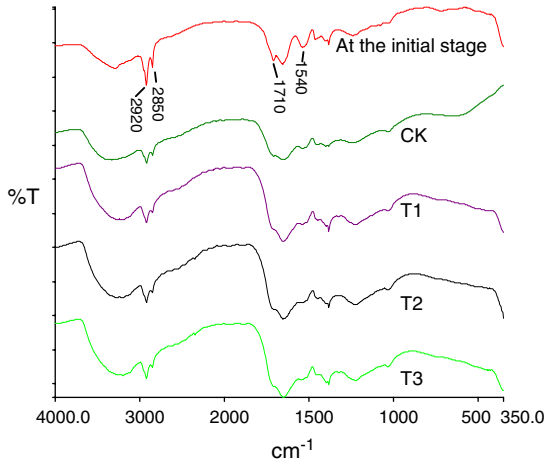

Fig. 3. HA FTIR spectra at the initial and final stages of MSW composting.

denced by the study (Chefetz et al., 1998) that the main changes in HA FTIR spectra with composting time are: (i) a reduction in, and transition to a small shoulder of, the 1716 cm^{-1} peak (COOH groups) in mature compost; (ii) a sharp decrease in the aliphatic region (2930 and 2850 cm^{-1}), and (iii) a relative increase in the aromatic peak (1650 cm^{-1}).

At the final stage of composting, the FTIR spectral pattern of HA for all the treatments were similar but the absorption intensities of certain characteristic peaks were different. This indicated that the HA formed similar structures, but that the structure unit and the quantity of functional groups were different as a result of the various treatments. In FTIR spectral analysis, a number of peak ratios have been used to monitor the chemical changes during composting (Inbar et al., 1989, 1991). In this study, at the final stage of composting, in comparison with CK, peak ratios for T1, T2, and T3 increased in the order: 1650/2920 (aromatic C/aliphatic C), 8.02%, 8.92%, 9.52%; 1650/1540 (aromatic C/amide C), 0.74%, 4.70%, 8.39; 1650/1030 (aromatic C/polysaccharides C), 4.54%, 12.80%, 26.09%. This indicated that the inoculated microbes could increase the aromatic content and decrease the polysaccharide and aliphatic contents of MSW composting. The extent of aromatization of the HA in the various treatments was in the order: T3 > T2 > T1 > CK. The changes indicate a loss of nitrogen-containing functions, alkyl chains and carbohydrates, which are used by the microorganisms to generate more condensed molecules. Similar variations have been reported by Garcia et al. (1992) and Baddi et al. (2003). Overall, inoculation would improve the degree humification and stable process for industrialized MSW composting.

3.3.3. Fluorescence synchronous scan spectroscopy

Fluorescence spectra in synchronous scan mode should allow better peak resolution than that from conventional fluorescence modes, i.e. emission or excitation. This provides a more accurate parameter for differentiating samples and a greater possibility for identifying the molecular structures responsible for the fluorescence in each sample (Senesi et al., 1991). The HA fluorescence synchronous scan spectra are shown in Fig. 4a. At the initial stage of MSW composting, the spectra exhibited a primary peak at 335 nm, a secondary peak at 450 nm, a minor peak at 475 nm, and a number of minor shoulders. At the final stage of composting, the HA fluorescence intensities decreased for all treatments, especially in the range of short wavelengths from 300 to 400 nm. In addition, at the final stage of composting, the fluorescence intensity of the secondary peak at 450 nm shifted towards the primary peak, from 450 to near 460 nm. The reason for this red shift was a significant degree of polymerization of multiple fluorophores, enhancing the probability of bimolecular processes such as radiative energy transfer and excimer/exciplex formation (Komada et al., 2002; Schepetkin et al., 2003). This may be attributed to a high degree of

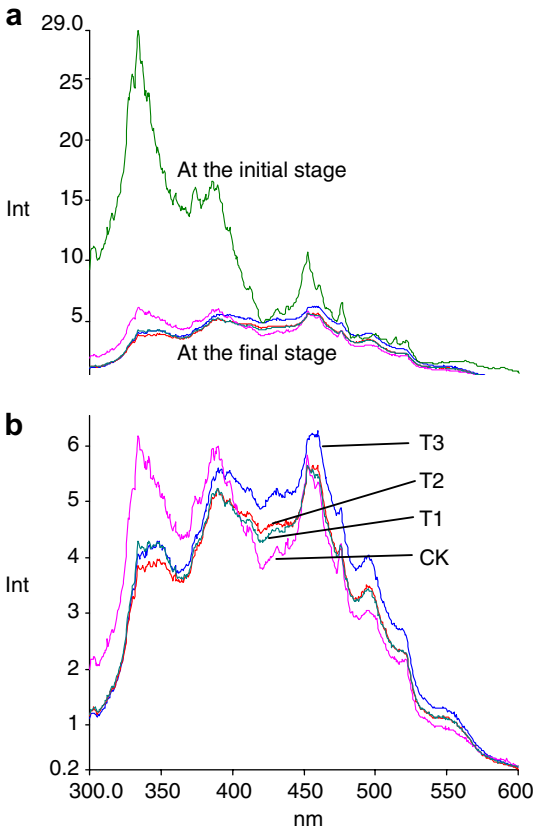

Fig. 4. HA fluorescence synchronous spectra during MSW composting: (a) at the initial and final stage composting; (b) at the final stage composting.

conjugation and the presence of many aromatic structures. Therefore, the result showed that the degree of HA humification increased at the final stage of MSW composting.

At the final stage of composting, the HA fluorescence synchronous scan spectra exhibited a number peaks at 335, 385, and near 450 nm in all the treatments (Fig. 4b), while the HA fluorescence relative intensities after different treatments revealed variations. The fluorescence intensities at 335, 385, and 450 nm were all similar in CK, while with microbial inoculation decreased at 335 nm as well as at 385 nm, compared with CK, and showed broad patterns. The spectra after inoculation exhibited a primary peak near 450 nm, thereby approaching the main peak at 460–480 nm for HA in soil (Senesi et al., 1991).

In general, the shortened wavelengths and high relative fluorescence intensities may be attributed to simple structural components, a low degree of aromatic polycondensation and a low level of conjugated chromophores. The main peak at long wavelengths would be associated with the presence of high molecular weight fractions in HA (Senesi et al., 1991). Therefore, the Fig. 4 showed that inoculating with microbes during MSW composting could increase the degree of polycondensation and aromatization of HA. This indicates inoculation microbes in composting would improve the humification process of composting. Furthermore, mixed inoculation with complex microorganisms and lingo-cellulolytic microorganisms could increase the degree of polymerization of HA, compared to that of samples inoculated with complex microorganisms or lingo-cellulolytic microorganisms alone.

Altogether, the results obtained from elemental analysis and spectroscopies (UV, FTIR and fluorescence spectroscopy) indicate that aliphatic structures diminish and aromatic structures are enriched in HA with microbial inoculation. Likewise, the microbial communities of inoculated treatments were at large level during composting process, compared to the treatment without inoculation. This implied that inoculation was able to improve biotechnological process and humification degree of composting. These results are in accordance with elemental and spectroscopes analysis. But comparing with soil HA, the behavior of the compost HA studied in this work indicates that this HA has the lower molecular size and aromatic condensation content because of short period of humification. These compounds are HA in neoformation which can be more easily biodegradable than soil HA. In this way, inoculation microbes could increase the degree humification for the industrialized composting process. When composts are used as a soil organic amendment, their HA in neoformation can be incorporated in the soil HA structure and contribute to chemical and physical fertility.

4. Conclusions

The elements analysis, UV, fluorescence and FTIR spectra of HA showed that inoculation with microbes during MSW composting could decrease the low molecular weight components (aliphatics, proteins, polysaccharides, etc.) and increase the degree of aromatization of compared with the HA when no inoculation was carried out. This suggested that the microbes could accelerate the compost maturation process. Also, mixed inoculation with complex microorganisms and ligno-cellulolytic microorganisms during composting had a clear advantage over inoculation with complex microorganisms or lingo-cellulolytic microorganisms alone. But comparing HA from the MSW compost to soil HA, the HA from compost present a lower degree of aromatization than that from soil.

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