

Article

Biolysed Sludge Composting for Nitrogen Conservation and Humification Improvements and Mechanisms

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Abstract: Aerobic composting of conventional municipal sludge has always had the problems of nitrogen loss and low humification. In this study, biolysed sludge (BS), polyacrylamids-added sludge (PS) and Fe (III)/CaO-added sludge (FS) were used for composting, respectively, and their effect on the physical-chemical parameters, nitrogen conversion and humification during composting were investigated. The results showed that the dissolved organic matter (DOM) concentration of the BS pile (23.1 ± 0.4 g/kg) was 48.4% and 48.4% higher than the PS (15.5 ± 0.4 g/kg) and FS piles (15.5 ± 0.0 g/kg) in the initial stage of composting and became the lowest after composting, suggesting that the degradation of DOM was promoted in the BS pile. BS can also increase the retention rate of total nitrogen (TN) ($27.8\% \pm 0.8\%$), higher than that in PS ($22.7\% \pm 1.1\%$) and FS ($24.6\% \pm 0.5\%$), which may be due to the lower production of ammonia nitrogen in the BS pile. Compared with PS and FS, BS provided more humic substance (HS) and humic acid (HA) for composting and the HA contents of the compost products were 34.4 ± 1.0 , 35.4 ± 0.2 and 34.0 ± 0.3 mg/g in the PS, BS and FS treated piles, respectively. Fourier transform infrared and the excitation-emission matrix revealed that BS and FS promoted the aromaticity and stability of HA. The degree of polymerization (DP) of the products from the BS (1.48) and FS piles (1.56) was higher than that of the PS pile (1.36). However, the germination index (GI) value ($133.4\% \pm 6.0\%$) of FS was lower than that of PS ($152.3\% \pm 6.2\%$) and BS ($158.3\% \pm 0.8\%$), showing that the products of FS composting contain more plant biotoxicity. Thus, compared with PS and FS, BS can increase the nitrogen retention rate and the maturity of the compost.

Keywords: biolysed sludge; compost; nitrogen conservation; humic acid; humification



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1. Introduction

With the fast development of industrialization and urbanization, daily wastewater treatment capacity has rapidly increased around the world [1]. As a by-product of the sewage treatment process, sewage sludge (SS) contains large quantities of pathogens, heavy metals and persistent organic pollutants that could do great harm to the natural environment and human society [2]. SS composting is an environmentally friendly and convenient technology which decomposes organic matter into a stable form in an effective manner and the compost products can be used as organic fertilizers or soil amendments [3]. The sludge conditioning pretreatment is an essential step to improve the dewatering efficiency of SS [4]. Fe(III)/CaO and polyacrylamids (PAM) are commonly applied as conditioners for sludge pretreatment, and PAM-added sludge (PS) and Fe (III)/CaO-added sludge (FS) are obtained, respectively [5]. However, composting with traditionally

dewatered SS generally meets some problems because of the chemical conditioner dosage. For example, FS is corrosive and the application of its compost products has degraded a large amount of cultivated land and brought economic losses. PAM can be degraded into toxic monomer acrylamide (AM) during composting which is harmful to the ecological environment [6]. Thus, it is necessary to find a sludge that does not contain chemical conditioners and is environmentally friendly to compost.

In recent years, *Bdellovibrio*-and-like organisms (BALOs)-based biocracking technologies have been developed for dehydration [7]. BALOs are widespread in manmade and natural environments such as lakes, oceans, soil, sewage and wastewater treatment plants [8]. They can selectively parasitize and can attack most Gram-negative bacteria without leaving residue or infection problems, and they do not attack eukaryotic cells which is safe for humans and animals [9,10]. Based on the utilization of BALOs' unique metabolic characteristics, Yu et al. previously developed an efficient, environmentally friendly and economical SS biolysis dewatering technology with no chemicals introduced, and obtained dewatered biolysed sludge (BS) with a water contents less than 60% [8]. Obviously, composting of BS could reduce environmental pollution caused by the addition of chemical conditioner dosages compared to FS or PS composting.

The major disadvantages of traditional SS composting are the low humification degree and nitrogen loss which hold back its industrial application [11]. Determining humification degree is essential for evaluating compost quality. Humic substances (HS) obtained by aerobic compost can be used to remediate soil and improve soil fertility and humic acid (HA) and fulvic acid (FA) are essential components of HS [12,13]. HA, which is composed of aliphatic and aromatic compounds, holds a high aromatic polymerization. The formation of HA can increase the aromatization and humification of HS and it is important to evaluate humification degrees [11,14]. The humification degree is affected by material properties and could be characterized by the content of humus and the molecular structure [15]. Zhang et al. found that the concentrations of HS and HA in chicken manure were higher than those in urea at 50 d of lignocellulose biomass composting [16]. Cao et al. reported that hyperthermophilic pretreatment composting could prolong the thermophilic stage and contribute to the formation of HA during composting [17]. The organic nitrogen in a composting mixture can be degraded to ammonia by ammonification, nitrified to nitrite and nitrate and will eventually be emitted into the atmosphere in the form of NH_3 , N_2 or N_2O by denitrification [18]. The emissions of N-containing gases will also reduce the agronomic value of compost products. Therefore, strengthening nitrogen fixation and retention in the composting process is key to promote SS treatment and recycling. Nitrogen conservation is mainly affected by material properties and other factors, such as microbial inoculation, carbon/nitrogen ratio (C/N) and pH [19]. Numerous researchers have shown that microorganisms play an important role in the transformation of substances during composting, which could control hazardous gas releases and limit nitrogen loss [20]. Hu et al. found that bioleached dewatered sludge used for composting drastically increased nitrogen conservation because of its lower pH, higher intensity of microbial assimilation and the presence of water soluble Fe compared to conventional sludge [19].

In the preliminary experiment (Figure S1), HS and HA in BS were found to be 50% higher than those in FS and PS, and this meant that BS composting might display great potential for HA generation and contribute to the degree of compost humification. A previous study found that biolysis strongly damaged the structure of sludge and the cell integrity, and partially degraded proteins in the extracellular polymeric substances [21]. It is still unclear how these changes in properties of the sludge affect nitrogen conservation during composting. It was speculated that BS composting could enhance nitrogen conservation because the relative abundances of the dominant genera *Ferruginibacter*, *Pseudomonas* and *Thermomonas* related to denitrification had a significant decrease after biolysis treatment [22]. However, published literatures have hardly documented composting of BS because studies on SS biolysis dewatering started just from 2017, as mentioned above. The objectives of this work are to understand the changes in the physicochemical properties of BS composting

and explore the potential of BS composting in enhancing humification degree and nitrogen conservation compared with those for traditional FS composting and PS composting. The findings of our study hope to provide guidance for the large-scale composting application of BS.

2. Materials and Methods

2.1. Sludge Conditioning and the Mechanical Dewatering of the Conditioned Sludge

PS was directly collected from one municipal wastewater treatment plant (MWTP) in Nanjing, China. In addition, the SS before dewatering was collected from a secondary sedimentation tank located in the same WWTP for Fe (III)/CaO conditioning and biolysis with BALOs, respectively. Specifically, the SS was mixed with CaO at 0.20 kg/kg dry mass (DM) in a 3000 L reactor equipped with a stirrer device for 10 min, and then FeCl₃ at 0.04 kg FeCl₃/kg DM was added into the above sludge. After stirring for 10 min, the sludge was dewatered with a plate-and-frame filter to obtain FS, while the BALOs cultures developed by [7,21] were inoculated into the same reactor and the sludge was aerated for 12 h and dewatered with the same plate-and-frame filter press to obtain BS.

2.2. Composting Treatment of Dewatered Sludges

Wheat straw (Surui Straw Processing Factory, Lianyungang, China) was cut into 1–3 cm pieces as the bulking compost agent, with physico-chemical properties shown in Table 1. The microbial inoculum for composting was purchased from Haowangnong limited company (Zhengzhou, China).

As shown in Figure 1, a 216 L stainless steel cubic box was developed as the compost reactor covered with a layer of foam board (4 cm in thickness) from outside and further covered with aluminum foil thermal insulators to reduce heat loss. Holes with diameters of 2 cm were evenly distributed at the bottom of the box, accounting for about 1/16 of the total floor area for natural ventilation and drainage. A removable foam lid cover was placed on top of the reactor for insulation.

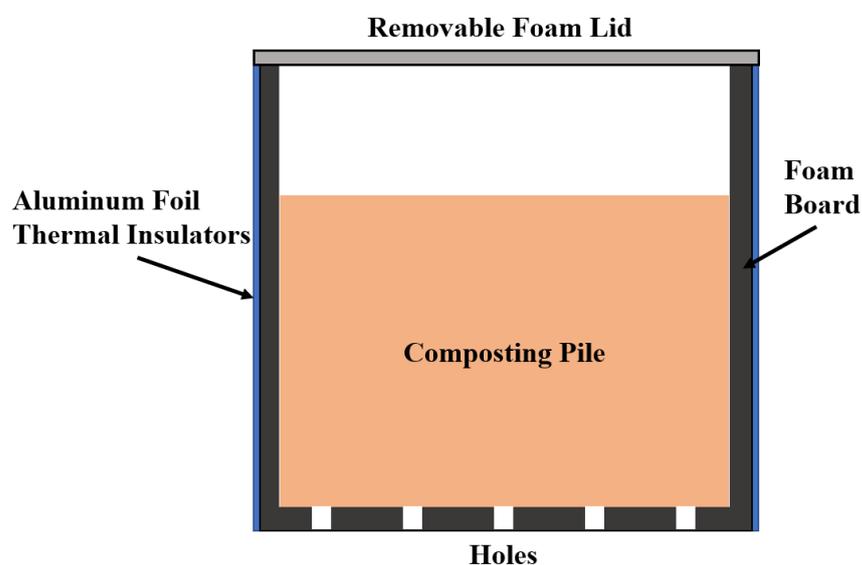


Figure 1. Diagram of the sludge compost reactor.

PS (15.9 kg), BS (7.2 kg) and FS (7.1 kg) (controlling the same mixed liquid suspended solids in dewatered sludge) were mixed with wheat straw (12 kg), respectively, to obtain a PS mixture, BS mixture and FS mixture. Fifty mL of Microbial inoculum was added to each mixture and the water content of each mixture was adjusted to about 58% by adding DI water. Eventually, the mass of each mixture was about 33.8 kg, and the volume of the mixture was about three quarters of the reactor. The composting piles were agitated

manually at certain intervals, i.e., three times per day in the first 6 days, twice a day from day 7 to day 15 and once every 3 days in the latter stage. The samples from different piles were collected before agitation on day 0, 2, 5, 8, 12, 17, 23 and 31 of the composting. Each sample was mixed by ten subsamples (approximately 120 g) from the depth of approximately 15 cm and 25 cm below the surface of the composting piles. The temperature and oxygen content (OC) of the composting piles were monitored twice a day before agitation with a mercury thermometer and oxygen monitor (Wanchuang electronic products Co., Ltd, Dongguan, China), respectively, at four different points 25 cm below the surface of the composting piles.

Table 1. Physicochemical properties of the raw composting materials.

	PS	BS	FS	Wheat Straw	PS Mixture	BS Mixture	FS Mixture
MC (%)	81.6 ± 0.0	59.1 ± 1.3	59.0 ± 1	6.2 ± 0.2	55.6 ± 2.3	54.1 ± 0.7	54.1 ± 0.5
OM (%DM)	48.3 ± 0.1	45.6 ± 0.3	41.2 ± 0.9	92.6 ± 0.1	84.9 ± 1.6	84.4 ± 0.9	77.3 ± 0.4
EC (µs/cm)	137 ± 11	214 ± 3	848 ± 51	1486 ± 72	1800 ± 52	1690 ± 8	2070 ± 33
pH value	6.94 ± 0.06	7.46 ± 0.06	9.12 ± 0.15	7.27 ± 0.03	7.35 ± 0.03	7.68 ± 0.13	7.89 ± 0.06
TOC (g/kg)	227.6 ± 2.2	207.5 ± 12.7	155.4 ± 21.2	387.5 ± 6.5	343.0 ± 5.3	339.3 ± 0.7	310.4 ± 1.8
TN (g/kg)	39.2 ± 0.8	36.3 ± 0.3	28.3 ± 0.4	4.7 ± 0.1	18.4 ± 1.2	16.1 ± 0.2	15.9 ± 6.5
C/N ratio	5.8	5.7	5.5	82.4	18.6	21.1	19.5

PS: PAM-added sludge; BS: biolysis sludge; FS: Fe (III)/CaO-added sludge; MC: moisture content; OM: organic matter; EC: electrical conductivity; TOC: total organic carbon; TN: total nitrogen; DM: dry mass.

2.3. Analytical Methods

The samples were divided into two parts. Part of the fresh one was mixed at a 1:10 (*w/v*) ratio with deionized (DI) water for 1 h at 30 °C and the mixture was filtered through a 0.45 µm filter membrane. An oxidation reduction potential (ORP) meter, pH meter, EC meter (Leici, Shanghai, China) and TOC analyzer (OI Analytical, College Station, TX, USA) were used to determine the ORP, pH, EC and dissolved organic matter (DOM), respectively [23]. A germination index (GI) test was conducted with the same water extract using cabbage seeds [24]. The moisture content was measured by weight loss of the compost sample after drying at 105 °C for 12 h and the OM was measured by burning at 550 °C for 6 h [20]. Fifty mL of 2 M KCl was used to extract 5 g of fresh sample and to determine the content of NH₄⁺-N, NO₂⁻-N and NO₃⁻-N in the piles [25].

The other one was crushed after air-drying and screened by a 100-mesh sieve, and then kept at −20 °C for further determination. The air-dried and crushed samples were oxidized by potassium dichromate to measure HS, HA and TN using the Kjeldahl method [26]. Loss of TN from the composting piles was estimated according to an equation by a previous study [27]. The extraction and purification of HS and HA were performed by referring to a previous method [23]. The solution of HA was divided into two parts: one part of the solution was diluted with DI water to a TOC of 10 mg/L for the determination of the three-dimensional excitation and emission matrix (3D-EEM) using a F-4600[®] fluorescence spectrophotometer (Hitachi, Tokyo, Japan) [28]. The other part of the solution was lyophilized to analyze the evolutions of the functional groups in the HA by Fourier transform infrared (FTIR) spectroscopy. A Nicolet iS-50[®] FTIR equipment (Thermo Scientific, Waltham, MA, USA) was used to determine the samples' characteristics, and the absorption bands ranged from 4000–400 cm⁻¹ [28]. The scanning analysis parameters were set as follows: monochromator slit widths of Ex = 10 nm and Em = 10 nm; scanning speed of 1400 nm/min; and wavelength range: Ex in the 200–600 nm and Em in the 200–600 nm [29].

The degree of polymerization (DP) was calculated according to Equation (1).

$$DP = \frac{C_{HA}}{C_{HS} - C_{HA}} \times 100\% \quad (1)$$

C_{HA} : carbon content of HA;

C_{HS} : carbon content of HS.

2.4. Statistics Analysis

All the samples were performed in triplicate and the data were presented as mean \pm standard deviations. Statistical analyses were carried out by Microsoft Excel 2020, Origin 2018. The significance of difference analysis was determined with SPSS 25.0 wherever applicable ($p < 0.05$).

3. Results and Discussion

3.1. Basic Physical and Chemical Indicators during Composting

The physicochemical properties of the sludge are presented in Table 1. Furthermore, FS exhibited the lowest OM, TOC and TN content among the three sludge types. This could be attributed to the addition of Fe (III)/CaO during the treatment process. In comparison to PS, the content of OM, TOC and TN in BS showed a slight decrease. This decrease may be attributed to the partial degradation of certain high-molecular-weight organic compounds in the BS, resulting in the formation of smaller molecular substances [21]. It can be observed that the MC of BS is comparable to that of FS, but significantly lower than that of PS ($p < 0.05$). The lower MC in BS suggests its potential to reduce the requirement for bulking agents in practical applications. Previous studies have demonstrated the use of bioleached dewatered sludge with a MC of 59.2% for composting, resulting in a reduction of approximately 10% of PS composting and saving around 700 kg of bulking agents per ton of sludge [19].

The processes of OM decomposition by microbes released heat to the composting piles. As shown in Figure 2a, the composting process of all three piles has gone through four stages: the mesophilic, thermophilic, cooling and mature stages. During the Mesophilic stage, the temperature rose sharply in the first two days and the FS pile reached a maximum temperature (68.4 °C) on day 2, which was higher than the 57.2 °C of the BS pile and 61 °C of the PS pile. Wang et al. found that lime can provide enough calcium to improve the metabolic activity of the microbial community, thus promoting the temperature of the composting piles [30]. At the same time, the reaction of the CaO residual in FS with water (moisture) also produced a certain amount of heat which may explain why the FS pile had the highest composting temperature. Although the maximum temperature of the BS pile was lower than that of the other two, it has been reported that thermogenic microorganisms could be killed when the temperature is too high and thus reduce the decomposition rate of the compost material [19], and this could be the reason that the BS pile still held a temperature above 50 °C for 7 days, which is longer than that of the PS pile (6 days) and the FS pile (6 days).

The BS pile had the lowest levels of OC during the mesophilic and thermophilic stages while the FS pile had the highest levels of OC (Figure 2b). Oxygen can provide an electron acceptor for the microbial biodegradation of OM. In general, the OC of the piles should be kept above 10 vol% during composting [31]. In the early stage of composting, although the agitation frequency was kept at three times a day, the levels of OC in the PS pile, BS pile and FS pile were maintained at $5.1\% \pm 0.1\%$, $3.3\% \pm 0.0\%$ and $4.1\% \pm 0.1\%$, respectively. This is probably because of the excessive oxygen consumption by aerobic microorganisms during the thermophilic stage [30]. The ORP values of the BS pile and the PS pile were both higher than that in the FS pile for most of the composting period (Figure 2c). Meanwhile, the ORP values of all three piles decreased first and then increased as the biochemical reaction process occurred, which is consistent with previous literature [32]. The higher levels of ORP in the BS and the FS piles indicated that the high oxygen diffusion in the composting matrix of the BS pile and the PS pile may increase oxygen consumption [33].

The FS pile held the highest pH during the entire composting period among all three piles, probably because of the contained $\text{Ca}(\text{OH})_2$. The initial pH values in the PS pile, BS pile and FS pile were 7.35 ± 0.04 , 7.68 ± 0.02 and 7.89 ± 0.01 , respectively (Figure 2d). All three piles were in the suitable pH range (5.5–8) for composting [19]. The pH rose rapidly in the early composting stage for all three treatments because of the production of $\text{NH}_4^+ - \text{N}$ (Figure 2a). At the end of the composting, the pH of the FS pile (7.59 ± 0.02) was

higher than that of the BS pile (7.02 ± 0.03) and the PS pile (6.57 ± 0.02). The formation of alkaline amino groups (C-NH₂) by humification or the retention of NH₄⁺-N could lead to the higher pH [11]. Considering the NH₄⁺-N concentration in the BS pile was lower than the PS pile, the main contribution of the higher pH value in the BS pile was biochemical humification rather than NH₄⁺-N.

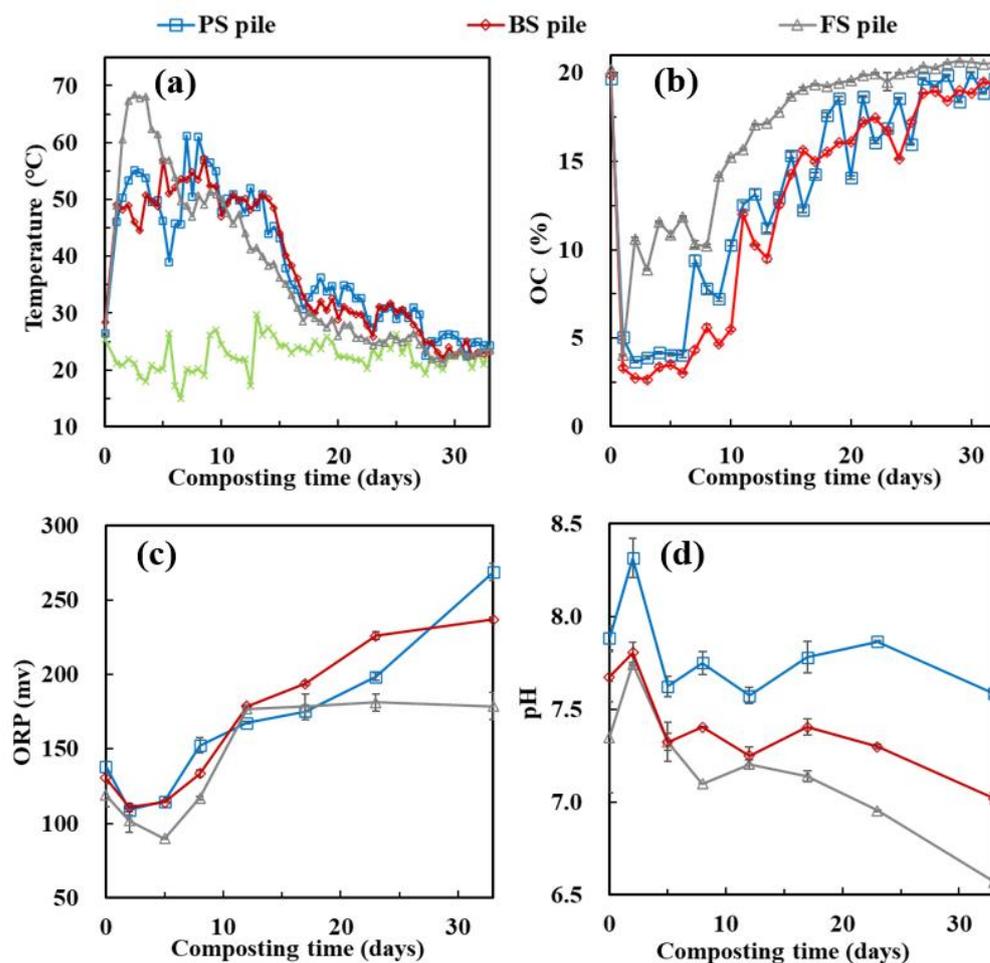


Figure 2. Dynamics of temperature (a), OC (b), ORP (c), pH and (d) during composting of the PS pile, BS pile and FS pile.

The composting product of the BS pile showed lower phytotoxicity than that from the FS pile or the PS pile. GI can reflect the degree of maturity of organic manure and the phytotoxicity of compost which is one of the most efficient biological indicators [34]. GI values of different sludge piles decreased slightly in the early composting stage (Figure 3a). From the eighth day, the GI values increased with the composting period. The final GI value in the BS pile ($158.3\% \pm 0.8\%$) was higher than that in the PS pile ($152.3\% \pm 6.2\%$) and the FS pile ($133.4\% \pm 6.0\%$). Studies have shown that the compost can be considered to be non-toxic to plants when GI is more than 80% [35], and the compost products from all three piles met this standard. EC can reflect the concentration of soluble salts in composting products and help determine phytotoxicity or inhibit plant growth [36]. The EC values of the three piles increased during the initial period (Figure 3b). After 33 d of composting, the EC in the BS pile (1.80 ± 0.04 mS/cm) was lower than that in the PS pile (1.88 ± 0.02 mS/cm) during the composting period, while the EC in the PS pile was significantly lower than that in the FS pile (2.34 ± 0.00 mS/cm) ($p < 0.05$). A previous study found that EC has a good correlation with GI, because numerous of the DOM and Soluble organic nitrogen which

affect the GI were produced with the degradation of the organics [37]. This may explain why the BS pile with lower DOM had a higher GI value than that in the PS and FS piles.

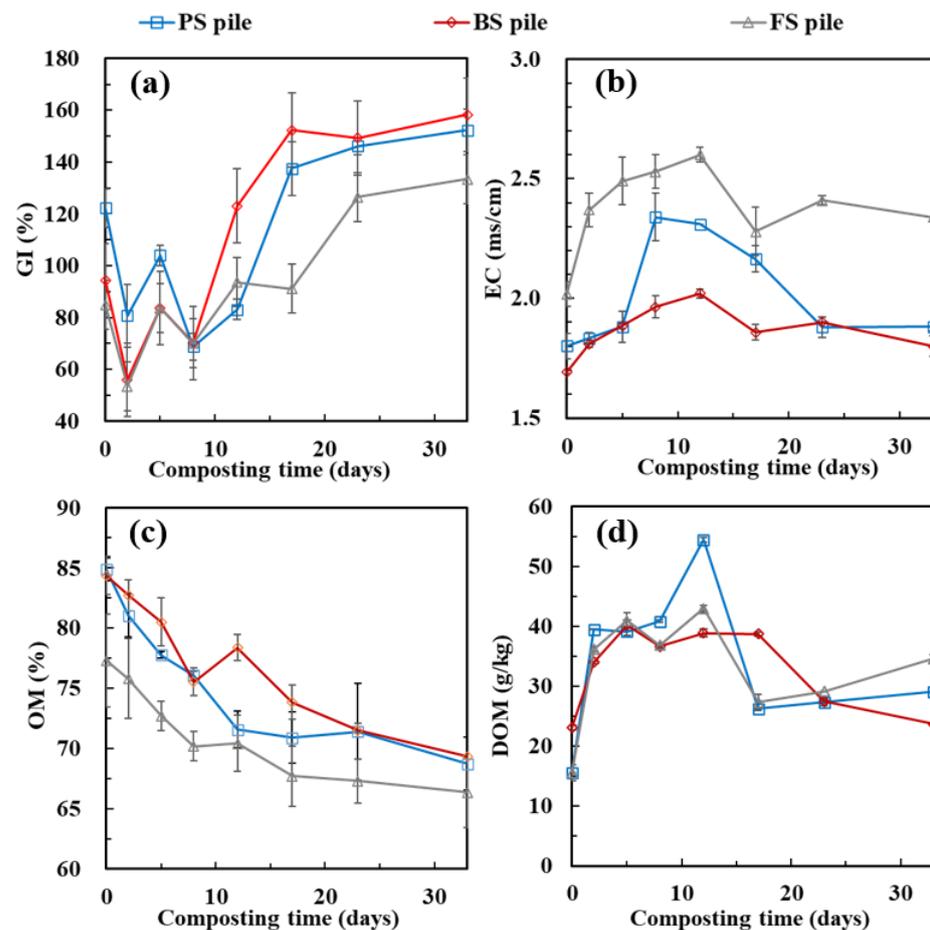


Figure 3. Dynamics of GI (a), EC (b), OM (c) and DOM (d) during composting of the PS pile, BS pile and FS pile.

3.2. Organic Matter Degradation

The OM content of all treatments showed a decreasing trend during composting and the FS pile had the lowest OM degradation rate (Figure 3c). The initial OM content in the PS pile, BS pile and FS pile were $84.9\% \pm 1.6\%$, $84.4\% \pm 0.9\%$ and $77.3\% \pm 3.8\%$, respectively. There was a distinct difference in the OM contents between the FS treatment and the BS and PS treatments, which was probably due to the inorganic substances, i.e., $\text{Ca}(\text{OH})_2$ and FeCl_3 , in the FS, reducing OM. After composting, the OM degradation rate in the BS pile (17.8%) was lower than that in the PS pile (19.0%), which may be attributed to the OM content partial degradation by biolysis in the BS treatment. Extracellular polymeric substances (EPS) were produced by cellular dissolution and adsorption of organic matter in WAS, containing 60–80% organic matter in the sludge, and part of the OM in the EPS was degraded in the process of the sludge biolysis treatment [21,38]. Partial degradation of the macromolecular organic substance will promote the release of a simple soluble OM from polymeric OM which may lead to an increase in DOM in the BS.

In OM, DOM has higher mobility and bioavailability, which can maintain microbial activity and nutrient availability in soil [35]. The initial DOM concentration of the BS pile (23.1 ± 0.4 g/kg) was 48% and 48% higher than that of the PS (15.5 ± 0.4 g/kg) and FS piles (15.5 ± 0.0 g/kg) (Figure 3d), showing that BS provides more DOM and it was easily consumed by microorganisms. This could also be the reason that temperatures above 50°C in the BS pile lasted longer (7 days) than that in the PS (6 days) and the FS

(6 days), which suggested that BS has a stronger heating and degradative potential. DOM increased during the initial composting days, which could be due to the degradation of organic macromolecules into small molecular substances and degradation of lignocellulose components [39]. After composting, the DOM content of the BS pile (23.7 ± 0.2 g/kg) was lower than the PS pile's (29.1 ± 0.0 g/kg) and the FS pile's (34.6 ± 0.6 g/kg), suggesting the degradation of DOM was promoted in the BS pile.

3.3. Nitrogen Conversion Index

With the rise in temperature, the NH_4^+-N concentrations increased rapidly during the first 2 days and peaked at 0.78 ± 0.07 g/kg in the PS pile, 0.58 ± 0.04 g/kg in the BS pile and 1.09 ± 0.06 g/kg in the FS pile, respectively, due to organic nitrogen ammonification (Figure 4a). Then, the concentrations of NH_4^+-N in the three treatments gradually dropped, probably due to nitrification or conversion into NH_3 [34]. At the end of the composting, the NH_4^+-N concentration of the BS pile (0.035 ± 0.007 mg/g) was lower than that in the PS pile (0.051 ± 0.009 mg/g) and the FS pile (0.048 ± 0.005 mg/g). It has been shown that GI has a distinctly negative correlation with NH_4^+-N concentration [40]. The concentration of NH_4^+-N produced in the BS pile was significantly lower than that in the PS and FS piles during the composting process and the lower ammonification rate in the BS pile implies that the BS contained less easily mineralizable organic nitrogen. Indeed, Yan et al. found that organic nitrogen such as protein in EPS were partially degraded after biolysis treatment [21].

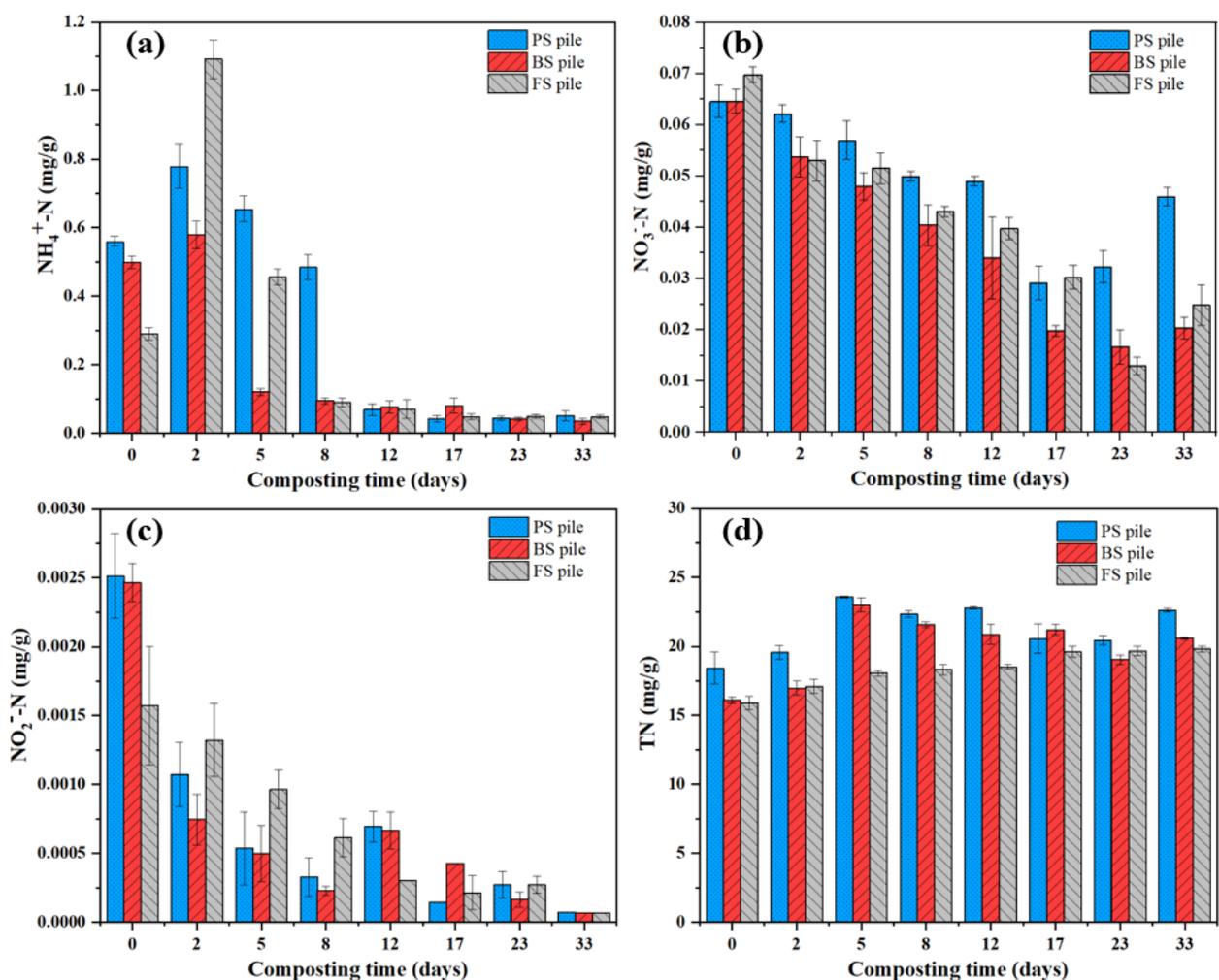


Figure 4. Changes in nitrogen-containing substances during composting: (a) NH_4^+-N , (b) NO_3^--N , NO_2^--N (c) and (d) total nitrogen (TN).

Nitrification is important to promote the conversion of $\text{NH}_4^+\text{-N}$ to oxidized N and reduce nitrogen loss [41]. Figure 4b,c shows that concentrations of $\text{NO}_3^-\text{-N}$ and $\text{NO}_2^-\text{-N}$ in the three treatments were lower than the concentrations of $\text{NH}_4^+\text{-N}$ during the whole composting stage, indicating that inorganic nitrogen mainly existed in the form of $\text{NH}_4^+\text{-N}$ in the composting process. From days 23 to 33, the $\text{NO}_3^-\text{-N}$ concentrations increased with the decrease in composting temperature, which was attributed to recovery of nitrifying bacteria [13]. $\text{NO}_2^-\text{-N}$ concentrations of all treatments were gradually decreased throughout the whole composting. As the first and rate-limiting step of nitrification, ammonification includes two processes: $\text{NH}_3/\text{NH}_4^+$ is oxidized to hydroxylamine, which is immediately translated to $\text{NO}_2^-\text{-N}$. $\text{NO}_2^-\text{-N}$ is later oxidized to $\text{NO}_3^-\text{-N}$ [42]. It was reported that the activity and growth of nitrifying bacteria could be inhibited because of the high temperature and numerous NH_3 [43]. Heterotrophic nitrifiers, of which the nitrification efficiency is normally lower than that of autotrophic nitrifiers, are often the dominant nitrobacteria [44]. At the same time, an anoxic microenvironment during mesophilic period could also lead to microbial denitrification [42]. Compared to the PS pile and the FS pile, the BS pile consistently held lower $\text{NO}_2^-\text{-N}$ and $\text{NO}_3^-\text{-N}$ concentrations during the composting period, which may be caused by lower $\text{NH}_4^+\text{-N}$ concentrations in the compost materials.

BS composting showed a minimum loss of TN among all three treatments, which indicated its enhanced nitrogen conservation potential. The TN content (per g of DM) in all three piles increased after composting. Specifically, the TN content of the BS pile and the PS pile exhibited an upward trend from day 0 to day 5 and day 22 to day 33 (Figure 4d), which could be attributed to the higher carbon decomposition rate than that of nitrogen and the reduction in compost mass [20,45]. The TN content in the BS pile and the PS pile decreased from day 5 to day 22 because of the bio-degradation of organic nitrogenous materials and the emissions of NH_3 [46]. At the end of composting, the TN contents in the PS, BS and FS piles were increased by $27.8\% \pm 0.8\%$, $22.7\% \pm 1.1\%$ and $24.6\% \pm 0.5\%$, respectively. The transformation of $\text{NH}_4^+\text{-N}$ in the composting mixture to ammonia gas is generally considered as the main route of nitrogen loss [47,48]. The lower content of $\text{NH}_4^+\text{-N}$ produced in the BS pile could be the reason that the nitrogen retention rate of the BS pile was 13.1% and 22.3% higher than the PS and FS piles, respectively (Figure 2d). Overall, the BS pile reduced the losses of TN by reducing the production of $\text{NH}_4^+\text{-N}$.

3.4. Humification Process during Composting

BS composting displayed better maturity in the final product among all three treatments. Converting biodegradable OM into HS and HA is a humification of organic waste composting [49]. The initial concentrations of HS and HA in the BS treatment were higher than those in the PS and FA treatments (Figure 5a,b). HA decreased during the mesophilic stage and was followed by a sharp increase in the BS pile and the PS pile. The decrease from days 0 to 2 could be attributed to the degradation of unstable HA with the temperature increasing. In general, as compost progresses, precursors such as polysaccharides and amino acids are converted to HS continuously. However, all treatments showed a declining trend in the cooling and maturing stages, probably due to the reduction in unstable compounds in HS [17]. After composting, the HA content in the BS pile (35.4 ± 0.2 mg/g) was higher than that in the PS (34.4 ± 0.4 mg/g) and FS (34.0 ± 0.3 mg/g) ($p < 0.05$) and the HS content in the PS, BS and FS piles were 59.8 ± 2.2 , 59.3 ± 0.9 and 55.8 ± 1.2 mg/g, respectively, implying that FA with low molecular weight was used as a nutrient substrate for microbial growth and was transformed into a more stable structure and larger molecular weight HA [50].

DP have been widely used to explore the effects of the humification process of composting [16]. As shown in Figure 6, DP increased throughout the composting which reflected the transformation from FA to HA [17]. After composting, the DP value in the BS pile (1.48 ± 0.01) and the FS pile (1.56 ± 0.02) was significantly higher than that of the PS pile (1.36 ± 0.04), indicating that products of the BS and the FS piles showed better maturity and stability than that in the PS. BS and FS may promote the polycondensation of the precursor.

There is an argument that humus behaves as an association of relatively small molecules rather than macromolecular polymers. Thus, the increase in pH of the BS and FS piles may contribute to the transformation of those small molecules from a low-molecular size fraction (FA) to high-molecular size fraction (HA) [51], which partly explains the higher DP in BS and FS during composting. That the FS pile had a slightly higher DP value may be because lime could promote the formation of precursors by contributing to the hydrolysis of C–H bonds [30]. However, considering GI was also an indicator for compost maturity and the GI of the FS pile was significantly lower than that of the BS, BS had a better maturity.

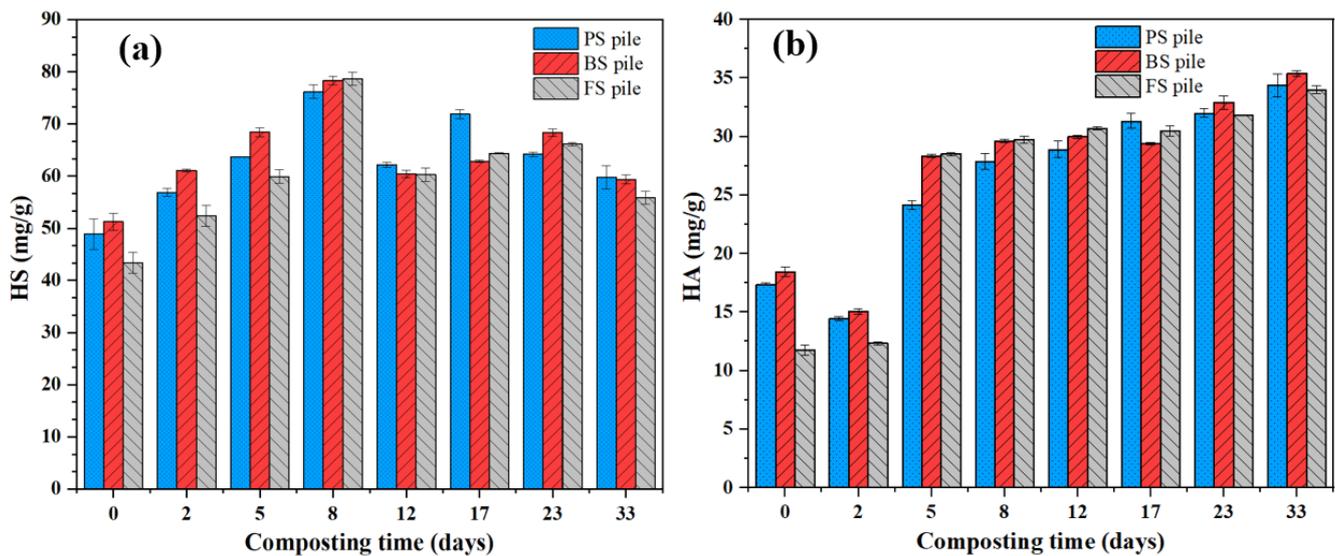


Figure 5. Changes in the extractable HS (a), HA (b) in PS, BS and FS pile during composting.

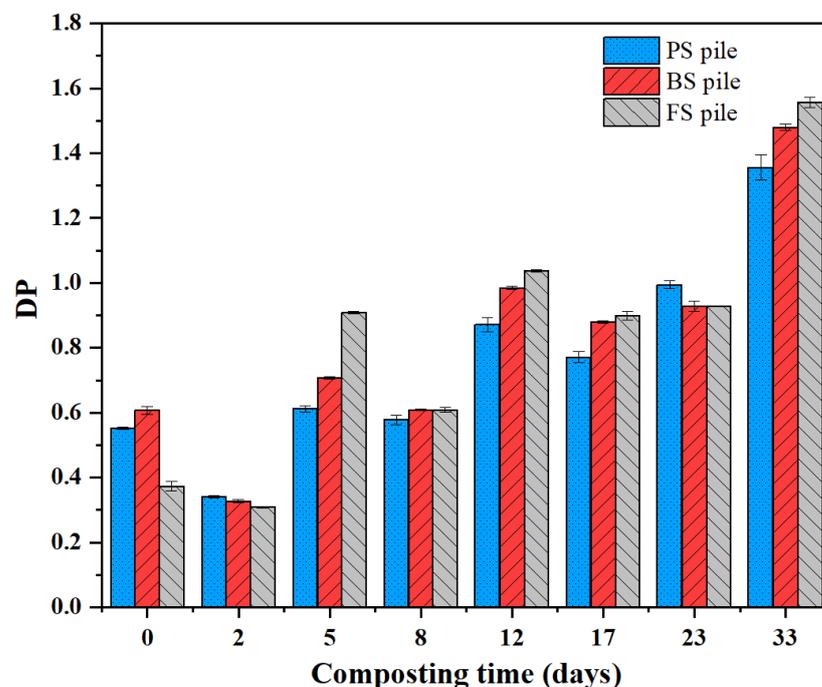


Figure 6. Changes of DP in PS, BS and FS pile during composting.

3.5. Fourier Transform Infrared Spectroscopy Analysis of Compost HA

BS and FS increased the aromaticity of HA which could increase the benefits of the composting end-products [52]. The spectra of HA had similar absorbance bands and vari-

ation trend at the onset and finish of the composting for the three treatments (Figure 7). According to the intensity, position and shape of the absorption peak in the FTIR spectra, the functional groups of HA contained in the composting mixture can be determined [53]. Previous literature [54] showed the absorption peak values and their corresponding functional groups (Table S1). The main absorbance bands were identified on the FTIR spectra for the HA of the three treatments (Figure 4). Peaks at 3401 cm^{-1} and $2927\text{--}2850\text{ cm}^{-1}$ represent the O–H stretching of alcohols or phenols C–H stretching of alkanes, respectively. Their decline in all treatments demonstrated that the microbes decomposed carbohydrate, fatty substances, polysaccharides, lignin and saturated hydrocarbons in HA and degraded with the progress of composting [51]. The peak intensities at 2958 cm^{-1} decreased due to the biodegradation of the complex molecules [55].

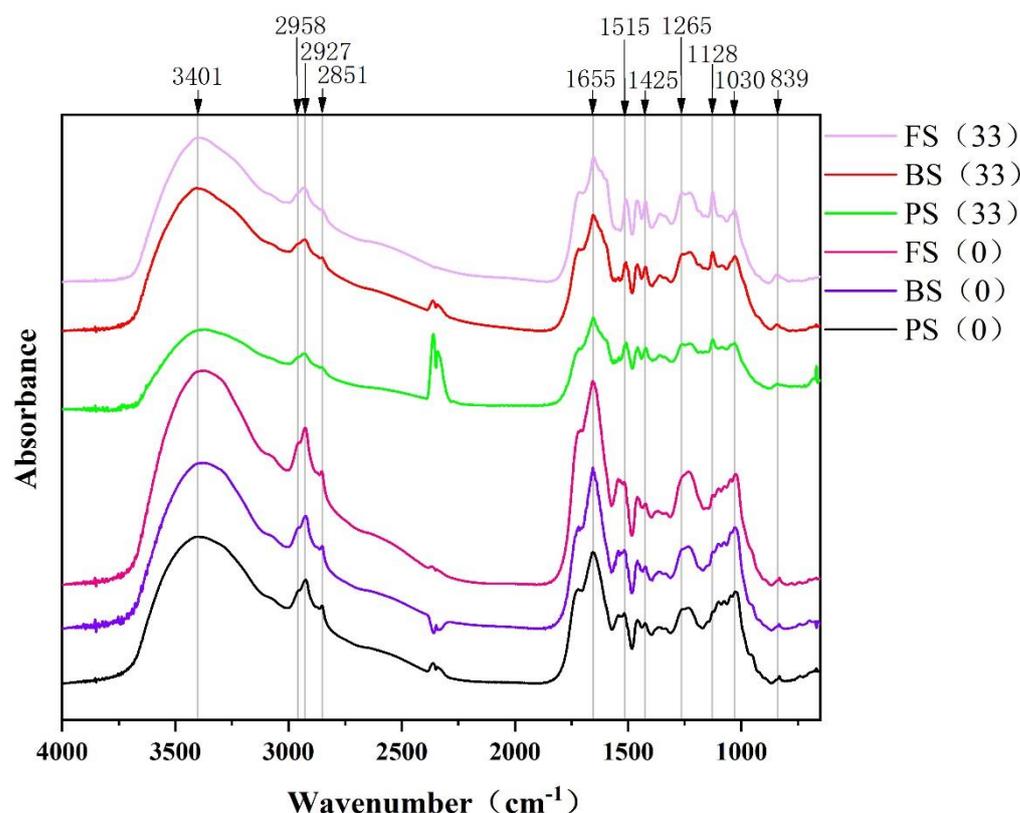


Figure 7. FTIR spectra of HA on days 0 and 33 in the PS, BS and FS pile.

The FTIR spectrum showed that the intensities of the absorption peak of 835 cm^{-1} , which is regarded as the C–H bending of the benzene ring or C–H bending of alkenes [51], in all three composting slightly rose during the processes, and the peak intensity in the BS and FS piles were higher than that in the PS pile, indicating that BS and FS promoted an increase in the aromatic ring and olefinic bond structure. The variation of intensity in 1665 cm^{-1} (C=C stretch of alkenes and aromatic rings) showing a similar trend to 832 cm^{-1} can also reflect the increase in aromaticity in BS and FS. In addition, stronger absorptions were found at 1030 cm^{-1} (aromatic ethers, –C–O–C of carbohydrates or Si–O–C groups) in the BS and FS piles after composting, suggesting that BS and FS could promote the polymerization of HA [55]. Previous studies have found that lime (existing in FS) could promote the formation of aromatic HS by inhibiting the activities of denitrifiers [52,56].

3.6. Fluorescence Characteristics of HA

HA is a fluorescent compound and fluorophore structure of compost-derived HA and is often analyzed by 3D-EEM [53]. According to a previous article [57], three-dimensional fluorescence spectrum can be divided into five regions and region I (Ex = 220–250 nm, Em = 220–330 nm),

II (Ex = 220–250 nm, Em = 330–380 nm), III (Ex = 220–250 nm, Em = 380–600 nm), IV (Ex = 250–550 nm, Em = 250–380 nm) and V (Ex = 250–550 nm, Em = 380–600 nm) were related to tyrosine, tryptophan, fulvic acid-like materials, soluble microbial byproduct-like materials and humic acid-like organics, respectively. It has been reported that the fluorescence intensity can reflect the degree of humification and aromatization of compost products [29]. After 33 days of composting, the fluorescence intensities of regions I, II and IV from all treatments had a slight increase (Figure 8). On the other hand, the fluorescence intensities of region III and V had a significant increase. The fluorescence intensities of humic acid-like organics in the BS pile were higher than that in the PS and FS piles on day 0, showing that not only raw sludge after biolysis can release more HA, but also improve the aromatic structures of the HA.

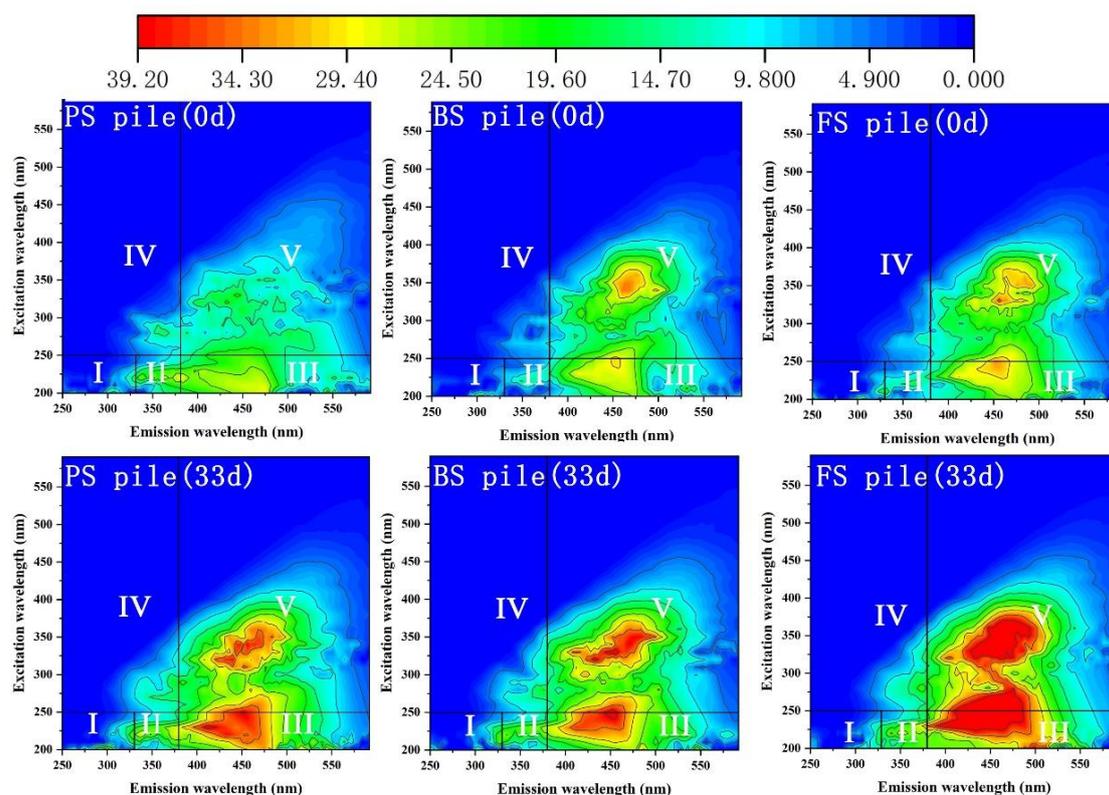


Figure 8. Three-dimensional excitation-emission matrix (3D-EEM) spectra of humic acid on days 0 and 33 in the PS, BS and FS pile. (Region I (Ex = 220–250 nm, Em = 220–330 nm), II (Ex = 220–250 nm, Em = 330–380 nm), III (Ex = 220–250 nm, Em = 380–600 nm), IV (Ex = 250–550 nm, Em = 250–380 nm) and V (Ex = 250–550 nm, Em = 380–600 nm)).

After composting, the fluorescence intensity of area III of the BS treatment was lower than that of the PS treatment while region V was the opposite, demonstrating that BS composting stimulated conversion from humic-like substances to a stabilized structure such as humic-like substances in HA. However, it is apparent that the intensities of region III and V in the FS pile were significantly higher than the BS and PS piles, which is consistent with the conclusion that lime can promote compost maturity [30]. In conclusion, the results of the FTIR and 3D-EEM spectra illustrated that the HA in the BS and FS piles contributed to a more intensive aromatization and stability than in the PS pile, demonstrating that BS and FS can promote the humification degree of compost, thus showing their potential in remediation of contaminated soils and may exhibit better stability in soil applications [11,54].

4. Conclusions

BS could provide more DOM (23.1 ± 0.4 g/kg) than PS (15.5 ± 0.4 g/kg) and FS (15.5 ± 0.0 g/kg) in the initial stage of composting and the degradation of DOM was promoted in the BS pile, which could increase the duration of the high temperature period. The lower ammonification rate in the BS pile implied that BS contained less easily mineralizable organic nitrogen and the lower content of NH_4^+ -N produced in the BS pile could be the reason that BS contributed to nitrogen conservation during composting. The FTIR and 3D-EEM results showed that BS and FS contributed to the increase in aromaticity and stability of HA, demonstrating that BS and FS can promote the humification degree of compost. After composting, the HA content in the BS pile (35.4 ± 0.2 mg/g) was higher than that in PS (34.4 ± 0.4 mg/g) and FS (34.0 ± 0.3 mg/g) ($p < 0.05$). The higher DP of the BS (1.48) and FS piles (1.56) showed that their compost products had a better maturity and stability than that in PS. The GI (133.4%) of the FS pile was lower than that in the BS pile (158.3%), indicating that the FS pile had higher plant toxicity. Therefore, BS composting has a higher nitrogen retention rate and maturity, showing a great potential in land use.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/su151310119/s1>. Figure S1: (a) initial contents of TOC and TN in PS, BS and FS, respectively; (b) initial contents of HS and HA in PS, BS and FS, respectively. Table S1: Main characteristics of the Fourier transform infrared (FTIR) spectra of HAs during different composting process.

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