THE RELATIONSHIP BETWEEN THE SPECTROSCOPIC CHARACTERISTICS OF HUMIC ACID IN SLUDGE COMPOST AND ITS ELECTRON TRANSFER ABILITY

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Abstract. Aerobic composting is the process of humification of organic matter, which produces a large amount of humus, and humic acid (HA) is one of the main components of humus. Ultraviolet-visible, infrared, and three-dimensional fluorescence spectroscopic techniques, combined with electrochemical methods, have been used to explore the changes in the structure and electron transfer ability of HA, which provide scientific evidence that compost products enter the soil. The results showed that during the first stage of fermentation, the polysaccharides, proteins, fats, and lignin in the HA are continuously decomposed, and that the degradation rates of the humus-like substances are greater than their generation rates, resulting in a decrease in their electron transfer capacity. Later, as the content of humus-like substances in the organic matter of HA increased, the content of aromatic substances such as quinones and phenols increased, and the degree of aromatization, humification, and stabilization increased, humification can accelerate the formation of redox-active substances in composting, and ultimately lead to the enhancement of electron transfer capacity.
Keywords: *humic acid, humus-like substances, spectral characteristics, aromatic compound, electrochemical methods*

Introduction

The essence of sludge composting is that the organic matter in the sludge is continuously mineralized and humified in its water-soluble phase under a redox reaction driven by microorganisms, which effectively eliminates pathogenic bacteria and reduces the volume of the sludge, finally resulting in the formation of a large quantity of stable humic substances (Kulikowska and Sindrewicz, 2018). However, since humus usually contains humic acid (HA), fulvic acid (FA), humin (HM), and other macromolecular substances, it is difficult to directly analyze its structure and composition. Since HA is one of the main components of humus, the mineralization and humification of the compost organic matter are mainly determined by its composition, the structure and composition of the HA can more effectively reflect the maturity of the compost. Therefore, the change in the structure and composition of HA is an important criterion for determining the compost quality and maturity (Rodriguez et al., 2016).

Studies have shown that humus has the ability to transfer electrons and has strong reversibility in the process of accepting electrons and supplying electrons, which can accelerate the conversion of heavy metals and organic pollutants by microorganisms (Aeschbacher et al., 2010; Piepenbrock et al., 2014). In recent years, scholars have begun to use electrochemical methods to study the electron transfer ability of dissolved organic matter (DOM) and solid-phase humus such as humin (Zhang and Katayama,

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2012; Yuan et al., 2013; Xiao et al., 2019), which may be applied in the reduction of heavy metal contaminants (Huang et al., 2010; Maurer et al., 2012), degradation of organic pollutants (Chen et al., 2011), manufacture of microbial fuel cells (Thygesen et al., 2009), and so on. Compost products can generally be used to repair soils and improve their quality (Wang et al., 2013); in addition, increasing the electron transfer capacity (ETC) of the compost products can enhance soil redox capacity and activate soil elements, thus accelerating the reduction transformation of pollutants. At present, most of the studies on the change characteristics of organic matter in sludge composting are focused on the change in the macrostructure of DOM of various composting materials. However, there is a lack of studies on the structure and properties of relatively single components, such as the HA, and there are only a few studies on the electron transfer ability of HA in sludge compost. Based on these facts, this study extracted HA samples in each stage of composting, uses spectroscopy and electrochemical methods to study their structural characteristics and changes in electron transfer ability, and provide a scientific basis for the production and regulation of good mature compost products.

Materials and methods

Aerobic composting system

The experiment was conducted at Guilin University of Technology in China. The municipal sludge was collected from Shangyao sewage treatment plant in Guilin City, with an initial water content of about 81% and organic matter content of 62%. Rice bran was purchased from a rice factory in Yanshan Town with an initial water content of about 9% and an organic content of about 89%. We used a self-made 50-L composting reactor comprising a fermentation tank, air pump, rotor flow meter, hose, and insulation material (*Figure 1*). The composting cycle was 30 days. The compost was set to use 15 kg dehydrated municipal sludge and 5 kg rice bran as auxiliary materials, which were mixed evenly and put into the reactor. In this study, we adopted the continuous forced oxygen supply mode, set the air aeration rate to 0.75 $L \cdot min^{-1}$, and wrapped the outside of the fermentation barrel with a thermal insulation material. The samples were taken at 0, 3, 6, 9, 14, 19, 24, and 29 days of the composting process.

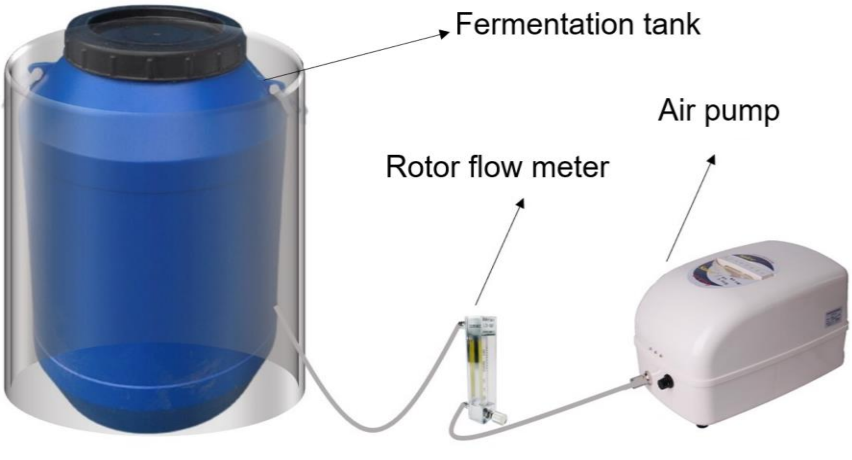

Figure 1. *Model of self-made experimental apparatus for aerobic composting*

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Experimental methods

HA was extracted according to the standard method provided by the International Humus Association (Lamar et al., 2014). To prevent the interference of organic carbon, the extracted HA was diluted to a certain total organic carbon (TOC) concentration, and the TOC concentration was obtained using the total organic carbon analyzer, it is manufactured by the Analytik Jena AG.

The ultraviolet–visible (UV-Vis) multiple spectra were obtained using the UV-6100A UV-Vis spectrophotometer, it is manufactured by Shanghai Yuanxi Instrument Company Limited. HA was diluted using ultrapure water until the TOC concentration was adjusted to 20 $mg \cdot L^{-1}$. Ultrapure water was set as the blank control, and the UV-Vis spectrum of the HA sample was obtained subsequently.

The three-dimensional fluorescence excitation-emission multiple spectra were obtained using the F98 fluorescence spectrophotometer, it is manufactured by Shanghai Lengguang Technology Company Limited. The three-dimensional fluorescence spectrum was obtained using the TOC concentration of the HA solution at 2 $mg \cdot L^{-1}$.

The Fourier-transform infrared (FTIR) multiple spectra were obtained using the CMT500A infrared spectrometer, used solid samples that have passed through a freeze dryer. The scan range was set to be 4000-500 cm^{-1}. The infrared spectrometer is manufactured by the American PE Company.

The traditional three-electrode circulation system was used to measure the electron-donating capacity (EDC) and electron-accepting capacity (EAC) of the HA components. with Ag/AgCl electrode as reference electrode, platinum column electrode as counter electrode, and a glassy carbon disk electrode as working electrode. The concentration of TOC of HA was maintained at 50 $mg \cdot L^{-1}$, and the ETC of HA was measured at CHI-660e electrochemical workstation, it is manufactured by Shanghai Chenhua Instrument Company Limited. The ETC is equal to the sum of the absolute values of EDC and EAC, which are calculated according to the method given in the literature (Tang et al., 2018).

Results and discussion

Change in temperature and pH during composting

Temperature is one of the most important parameters in aerobic composting. The change in temperature occurring during composting is shown in *Figure 2(a)*. The composting experiment was started in August 2020, during which time both the indoor and outdoor temperatures were relatively high. In addition, the products formed from the artificial mixture were loose and the pile body also heated up quickly. The highest temperature was close to 65°C. During the high-temperature period that lasted for 8 days, temperatures reached 50°C and above, and a second fermentation process occurred during the maturity period. It can be seen in *Figure 2(a)* that the temperature rose from 14 to 19 days, indicating that the compost material underwent secondary fermentation. Therefore, the compost was divided into the main fermentation period (1-14 d) and the second fermentation period (14-29 d).

The change in pH occurring during composting is shown in *Figure 2(b)*. The rise in pH was found to be faster during the early stage of composting, which may be due to the availability of sufficient oxygen during the early stage. The large amount of NH_4^+-N produced by the massive multiplication of ammoniating bacteria and the deamination of

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organic matter during the heating process had a certain contribution toward the weak alkaline nature of the composting mixture. The gradual decomposition of some of the organic acids in the sludge also led to the rise in the pH value of the entire composting process.

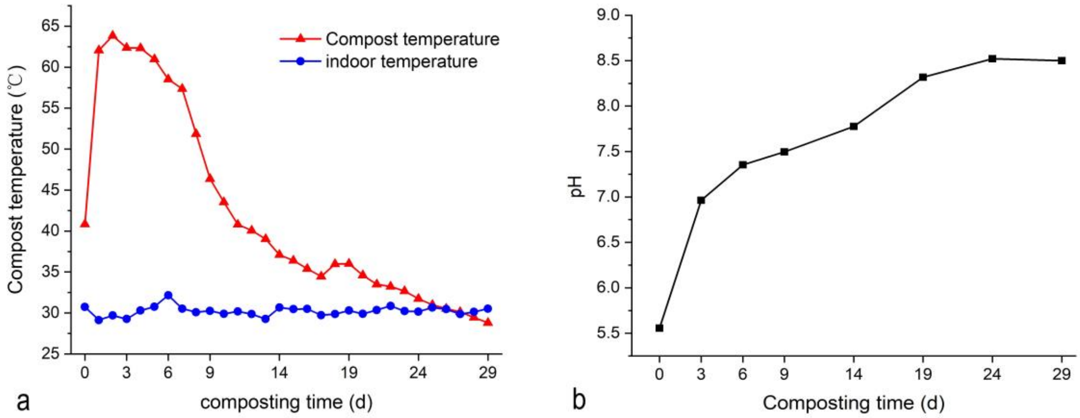

Figure 2. *The temperature change pattern during the composting process (a); The pH change pattern during the composting process (b)*

Analysis of the UV-Vis spectra of HA

UV-Vis spectroscopy is often used to characterize the structural characteristics of compost organic matter because of its easy application, fewer sample requirement, and high sensitivity. The UV spectrum of HA is shown in *Figure 3*. It can be seen from the figure that the absorbance of HA decreases with the increase in the wavelength. The UV-Vis curve has no obvious absorption peak, with only a shoulder peak appearing at 250 nm. This is due to the $\pi \rightarrow \pi^*$ transition of electrons in the aromatic compounds containing π-π conjugated structure, found in HA. With the increase in the composting time, there is a gradual upward shift of the UV-Vis curve, which shows that the concentration of humus-like substances in the HA organic matter continues to increase.

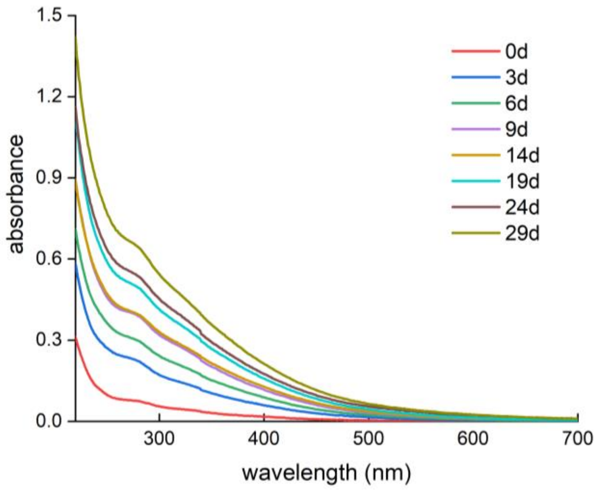

Figure 3. *UV-Vis spectra of HA at each stage of composting*

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The characteristic value of the spectral wavelength in the UV-Vis spectrum can reflect a lot of important information. Generally, A_{254} (the absorbance value of the organic matter at 254 nm) is used to characterize the content of C=C double bonds, which is represented by $SUVA_{254}$ (A_{254}*100/TOC) (Albrecht et al., 2011). The higher the value, the higher the content of unsaturated compounds containing C=C double bonds in the aromatic compounds. As shown in *Figure 4(a)*, The $SUVA_{254}$ value of HA was found to increase from 0.45 to 3.68 during the initial to the last stage of the composting process, which means that the content of the C=C double bonds of HA increases with increasing compost time, and that the HA aromatization and humicization constantly increased. The $SUVA_{280}$ (A_{280}*100/TOC) value is often used to measure the degree of humicization of the organic matter. The $SUVA_{280}$ value positively correlated with the molecular weight of the organic matter. The $SUVA_{280}$ value of HA increased from 0.38 to a maximum of 3.22, which indicated that the macromolecular organic matter content increased during composting and that the degree of agglomeration deepened constantly (Chin et al., 1994). This is because microorganisms are easier to degrade and utilize simple molecular substances such as sugars and proteins in the early stage, enabling macromolecular substances to gradually accumulate; in addition, simple compounds such as amino acids and lipids will aggregate into high-humification macromolecular substances. The change trends of $SUVA_{254}$ and $SUVA_{280}$ are basically the same, which reflects that the increase in the aromaticity of HA is related to the increase in the content of macromolecular substances.

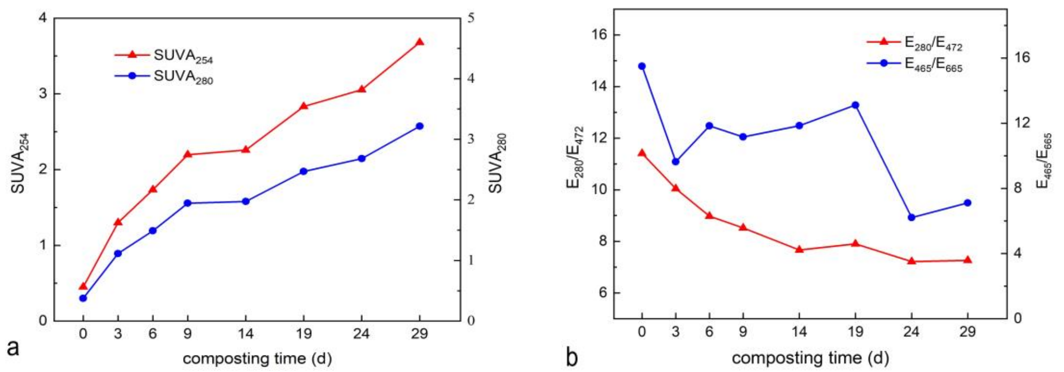

Figure 4. *Changes of $SUVA_{254}$ and $SUVA_{280}$ of HA in each stage of composting (a); Changes of E_{280}/E_{472} and E_{465}/E_{665} of HA in each stage of composting (b)*

In addition to the characteristic value of the spectral wavelength, the characteristic absorbance ratio is also often used to indicate the maturity and humification degree of the compost. The ratio of the absorbance values at 280 and 472 nm (E_{280}/E_{472}) can effectively indicate the relative content of lignins. The smaller the E_{280}/E_{472} ratio, the lower the relative content of lignins, the higher the relative content of HA, and the higher the degree of humification of the compost. It can be seen from *Figure 4(b)* that the relative content of HA lignin decreases during the composting process, and that the degradation rate during the early stage is faster; however, the observation that there is basically no change after 14 days indicates that the degradation of lignin mainly occurs during the primary stage of fermentation. This is because microbes are active during the

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early stage of composting, and lignin substances are one of the reaction substrates for microbial decomposition and polymerization of macromolecular aromatic substances.

The E_{465}/E_{665} value is the most common parameter for judging whether the compost stabilizes. It can reflect the degree of polymerization of the carbon skeleton in the benzene ring and is negatively correlated with the molecular weight of the organics and the degrees of polymerization and aromatization (Li et al., 2014). It can be seen from *Figure 4(b)* that the value of E_{465}/E_{665} fluctuates greatly and shows a downward trend in general, especially during the second stage of fermentation of 19 days. This is because the aromatization and humification of compost mainly occur during the second stage of fermentation. A large number of macromolecular aromatic compounds, such as quinones and phenols, were also formed during this period, which is consistent with the observation of the above characteristic parameters. The molecular weight of the HA components increased significantly during the whole composting process, and the organic matter with a more complex structure and a more stable and higher humification degree was formed under the action of microorganisms.

Evolution of HA components based on fluorescence spectroscopy

As shown in *Fig. 5*, to obtain more details about the characterization of compost-derived HA, three fluorescent components characterized by Ex/Em-365/485 nm (component 1, C1), 325/395 nm (component 2, C2) and 285/340 nm (component 3, C3), were identified by excitation–emission matrix-parallel factor analysis (EEM-PARAFAC). The PARAFAC can effectively avoid the overlapping of fluorescence peaks and decompose the three-dimensional fluorescence data matrix into individual fluorescence components, different types of organic compounds correspond to characteristic fluorescence components respectively, so as to conduct qualitative and quantitative analysis of organic compounds.

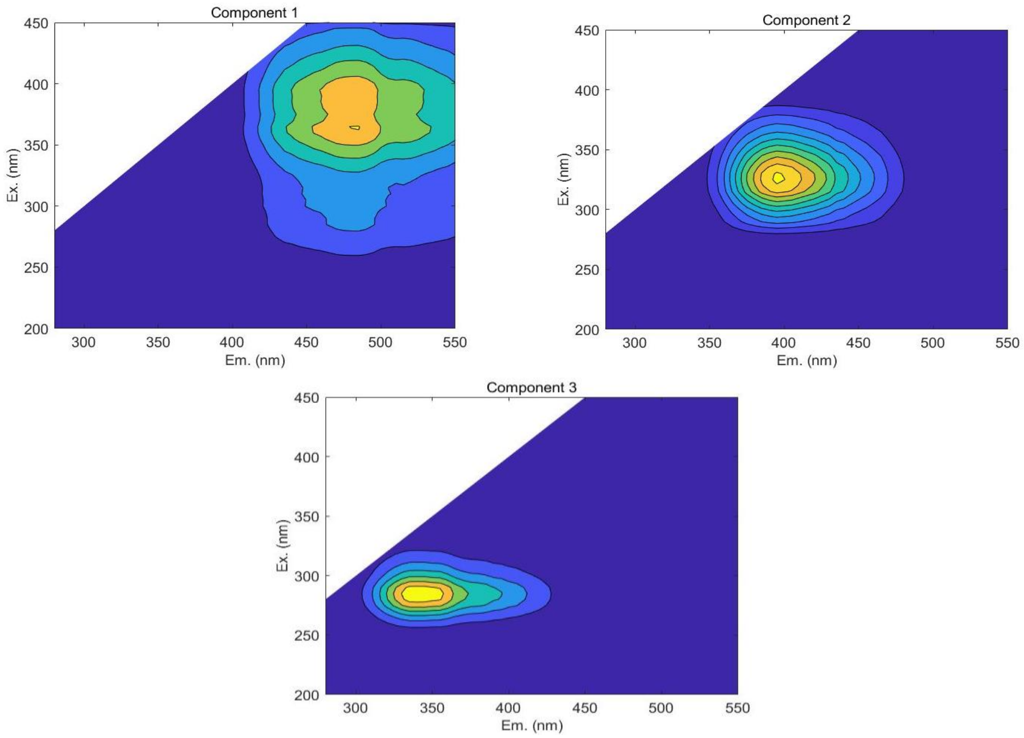

Figure 5. *Three fluorescence components identified by PARAFAC*

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According to previous reports (He et al., 2014; Yu et al., 2019; Huang et al., 2021), it is taken that C1 is related to humic-like substances, C2 represented fulvic acid-like substances, C3 represented soluble microbial by-products and tryptophan, it mainly includes small molecular substances such as protein, coenzyme and small organic acid.

The maximum fluorescence intensity (*Fmax*) was obtained by PARAFAC analysis, which represented the relative percentage content of certain fluorescent components in the sample. As shown in *Fig. 6*, in the initial phase, the protein-like substances represented by C1 are the main constituents of organic compounds in HA, and it can be seen that obvious protein degradation occurred with the composting process. In addition, under the action of microorganisms, proteins also generated humus-like substances with larger molecular weights and more complex structures through condensation reactions. Therefore, the content of C1 decreased and the content of C3 increased steadily. The Fmax value of C2 reached its maximum on day 6 and then decreased over the following days. FA is a precursor to the synthesis of HA, the decreasing trend of C2 in the humification stage could be explained either by the formation of the humic acid-like fraction as a result of polymerization of the fulvic acid-like fraction, or by the biodegradation of the fulvic acid-like fraction due to its nonhumic or easily decomposable characteristics (Yu et al., 2019).

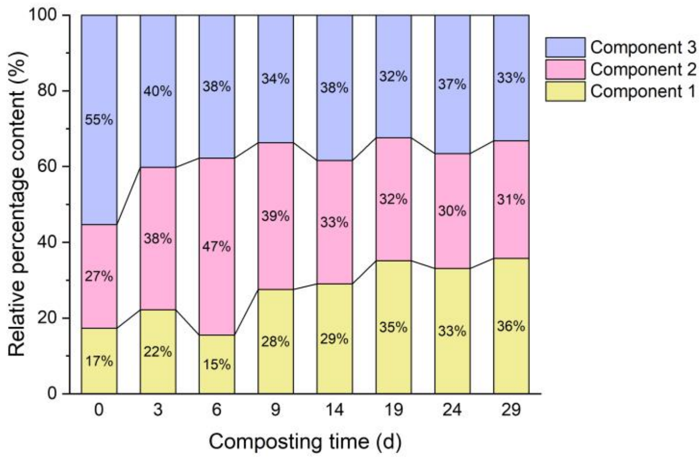

Figure 6. *Fmax of the three fluorescent components identified by PARAFAC*

Evolution of the HA functional groups based on FTIR spectra

FTIR spectroscopy can effectively characterize organic functional groups. The FTIR spectrum of HA is shown in *Figure 7*. It can be seen that the sample has absorption peaks near the wavelengths 3436, 2925, 2854, 1658, 1540, 1454, 1384, 1241, and 1049 cm^{-1}. It can be seen from the figure that the absorption peak at wavelength 3436 cm^{-1} is more broad, corresponding to the stretching vibrations of phenols and the hydroxyl group of alcohols (Marouani et al., 2019). The vibration frequencies at 2925 and 2854 cm^{-1} correspond to the stretching vibrations of the CH_2 group in the aliphatic structure (Jia et al., 2013), the absorption peak at 1540 cm^{-1} corresponds to the N-H, C-N, and C=N stretching vibrations of amino compounds such as amides (Peltre et al., 2017), and the peak at 1049 cm^{-1} corresponds to the C-OH stretching vibration of the polysaccharides (He et al., 2011). It can be seen from *Figure 6* that the intensities of these four absorption peaks are continuously reduced during the first period of

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fermentation of 0-9 d and that there is basically no change after entering the second period of fermentation of 14 d. It shows that the polysaccharides, proteins, and lipids of HA are continuously reduced during the main fermentation process of composting, which is consistent with the conclusion drawn by fluorescence spectroscopy. There is also a slowly increasing absorption peak at 1658 cm^{-1}, which is caused by the C=O of ketones and quinone carbonyl or the C=C stretching vibration of the aromatic ring group, indicating the increase in the concentration of the aromatic substances such as quinones (He et al., 2013). The peak at 1241 cm^{-1} is derived from the C-O stretching vibration of ester or ether. The absorption peaks at 1454 and 1384 cm^{-1} is derived from the asymmetric and symmetric bending vibrations, respectively, of the CH_3 group. However, the absorption peak at 1384 cm^{-1} appeared only during the first 9 days; it then disappeared, and a new absorption peak appeared at 1401 cm^{-1} on the 14th day and continued to increase. This is caused by the bending vibration of the CH_2 group on the olefin end group, which also shows that the structure of the compost in the second stage of fermentation becomes gradually complex and that the degree of compost maturity intensifies.

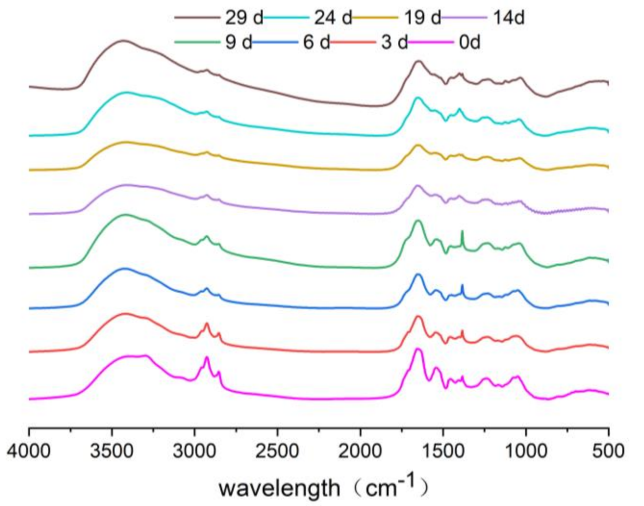

Figure 7. *Fourier Infrared Spectra of HA at each stage of composting*

Evolution of electron transfer capability

Humus has a large number of functional groups with redox characteristics, which can result in its conversion from the reduced state to the oxidized state and mediate the transfer of electrons between the microorganism and the electron acceptor (Kluepfel et al., 2014). Studies have found that extracellular respiratory bacteria use humic substances as electronic shuttles, which can effectively promote the degradation of nitrobenzene and dechlorination of pentachlorophenol (Yuan et al., 2017). The results are shown in *Figure 8*, the EDC and EAC of HA have a similar trend; that is, they first decrease and then start increasing gradually. The increase in EDC was more than that in EAC, it shows that the reducing ability of HA is stronger than its oxidizing ability. EDC increased from 168.56 $\mu mol \cdot gC^{-1}$ during the initial stage of composting to 336.35 $\mu mol \cdot gC^{-1}$, an increase of 99.5%; EAC increased from 205.48 $\mu mol \cdot gC^{-1}$ at the beginning to 280.32 $\mu mol \cdot gC^{-1}$, an increase of 46.03%; This is because the microbial

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degradation rate is fast and the chemical reaction is intense during the main fermentation period of compost, and the organic matter is in a state of dynamic balance. After the degradation of protein-like substances and carbohydrates, the degradation rate of humus-like substances under microbial action is greater than the generation rate, resulting in the reduction of electron transfer capacity. Some studies (Tuomela et al., 2000) proposed that, in the process of humification of organic matter, lignin-like substances would be transformed into phenolic compounds and slowly oxidized into quinone compounds. In addition, the high-site active functional groups, such as quinones and phenols, in the aromatic compounds are the main contributors to the electron transfer ability of organics (Scott et al., 1998; Cory and McKnight, 2005; Walpen et al., 2016). In the second fermentation stage of composting, the aromatization and humification of organic matter led to the increase in the redox-active functional groups on the surface of HA, and the content of quinone and phenol groups, because of which the ETC is enhanced.

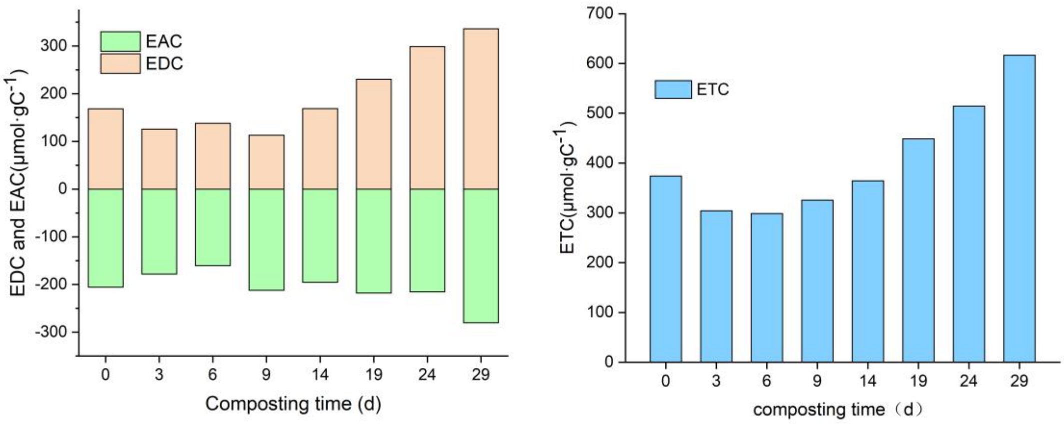

Figure 8. *The EDC, EAC and ETC change patterns of HA in each stage of composting*

The relationship between ETC, fluorescence characteristics and UV characteristic parameters

Correlation analysis was used to elucidate the relationship between ETC, fluorescence characteristics and *UV* characteristic parameters. We used IBM SPSS Statistics 25 to calculate the Person correlation coefficient among each parameter index，As shown in the *Fig. 9*, the C1 of HA was positively correlated with its ETC ($p<0.05$), and showed no obvious relationship with the other components, suggesting that the formation of humus-like substances moieties facilitated improvements in the ETC. Furthermore, the $SUVA_{254}$ and $SUVA_{280}$ were positively relative to C1 ($p<0.01$), this also indicating that C1 is mainly composed of unsaturated aromatic substances with high molecular weight. These aromatic compounds represented by quinones were the major contributing groups to ETC. In addition, E_{280}/E_{472} was negatively correlated with the C1 ($p<0.05$), $SUVA_{254}$ and $SUVA_{280}$ ($p<0.01$), This is because the lignin-like substances are precursors of humus substances, the decrease of lignin-like content indicated that lignin-like substances were gradually converted into macromolecular humus during composting. Therefore, humification can accelerate the formation of redox-active substances in composting, and ultimately lead to the enhancement of ETC.

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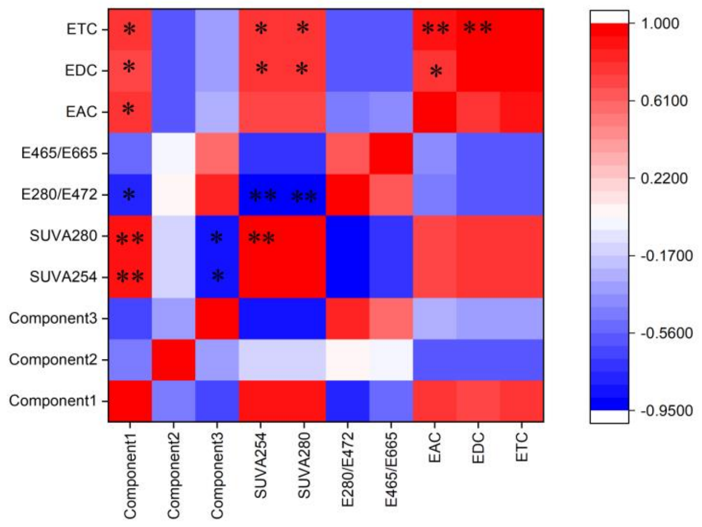

Figure 9. *Correlation analysis between ETC, fluorescence characteristics and UV characteristic parameters. *Correlation is significant at the 0.05 level, **Correlation is significant at the 0.01 level*

Conclusions

Modern spectroscopic techniques combined with electrochemical methods were used to study the characteristics of HA in sludge compost. The results of UV-Vis spectroscopy showed that the content of lignins in HA decreased during one fermentation, and the enhancement of the values of $SUVA_{254}$ and $SUVA_{280}$ indicated that the humification, aromatization, and molecular weight of HA continue to increase. Both three-dimensional fluorescence spectroscopy and FTIR spectroscopy show that during the main fermentation of the compost, the small molecular substances such as polysaccharides, proteins, and lipids in HA are continuously degraded under the action of microorganisms. The mineralization and humification of HA mainly occur during the second fermentation period after 14 days. The content of aromatic compounds, such as quinones and phenols, also increases at this stage, which results in the formation of HA with more complex structures and higher molecular weights, the ETC of HA is enhanced after humification, which has a positive effect on improving soil redox capacity and accelerating pollutant degradation.

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