Original Research

Dynamic Changes of Humic Acids in Chicken Manure Composting

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Abstract

In this paper, composting technology was used to treat agricultural waste chicken manure. The dynamic changes of the relative amount, chemical, optical and thermal properties of humic acid during the composting process were systematically studied. The results showed that the relative amount of humic acid increased from 5.46% to 8.36% with the ratio of H/C decreased and the condensation degree of humic acid molecule increased. In the infrared spectrum of humic acid, the integral area ratio of aromatic absorption peak to aliphatic absorption peak decreased from 0.51 to 0.14, and the lgK also decreased, it indicated that composting treatment was beneficial to the decomposition of simple compounds and the formation of complex organic matter in chicken manure. However, after 30 days of composting treatment, the lgK decreased to 0.85, which was still higher than that in farming soil, i.e., the molecular structure of humic acid in chicken manure treated by composting still has potential for further stabilization. Differential thermal analysis showed that the Peak Exothermic Temperature and weight loss all increased with the increase of composting time of chicken manure, which indicates that the molecular structure of humic acid in chicken manure became more complex and the degree of humification was obvious. Therefore, composting treatment can not only increase the relative amount of humic acid in organic materials but also improve the quality of humic acid.

Keywords: composting of chicken manure, HA, element composition, spectral characteristics, thermal properties

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**Introduction**

With the development of chicken large-scale and intensive culture in China, the supply of eggs and chicken meat has greatly increased, while the production of chicken manure has also increased. Under the influence of various reasons such as the labor cost and transportation cost, the proportion of producing organic fertilizer with poultry manure and other livestock manure as raw materials is not high. A large number of excess livestock manure such as chicken manure, which has great potential environmental pollution risk [1, 2]. At present, livestock and poultry manure composting are one of the best ways to effectively avoid the potential environmental pollution risk of livestock and poultry manure. The composting process is not only a process of mineralization and decomposition of organic carbon in livestock and poultry manure, but also a process of simple organic compounds re-synthesizing humus, and the formation of macromolecule organic matter humus in chicken manure composting process. The organic matter humus formed in the process of chicken manure composting is an important active substance constituting soil fertility, therefore, the nature of humus matter in the process of chicken manure composting directly affects the quality of composting [3].

Humus generally includes humic acid, fulvic acid, and humin. Humic acid (HA) is a series of macromolecule condensates with complex structure, quinone structure, and phenol structure, and different molecular weight [4]. It belongs to a kind of organic colloid with variable charge and contains many functional groups, such as carboxyl group (COOH), Phenolic-OH, Ketonic-C=O, Quinonid-C=O and Alcoholic-OH which have strong chelating ability [5]. At the same time, humic acid is also the most abundant and stable component of soil organic matter, with loose ‘spongy’ structure and huge surface energy. Its morphology, molecular composition, and physical and chemical properties have a significant impact on the fertility characteristics of organic fertilizer.

Smidt et al. simulated aerobic composting process in the laboratory and found that adding lignin could improve the formation of humic acid in the composting process [6]. According to FTIR and thermal analysis results, it was found that part of the added lignin has become a part of the molecular structure of humic acid in composting. Therefore, how to improve the content of humic acid and the stability of humus in composting process has been attracted attention widely. From the complexity of the research methods, the accuracy and timeliness of the data obtained, the analysis and determination of humic acid in the composting process are better than that of fulvic acid and humin. Consequently, it will be of great significance to study the changes in the quantity and structure of humic acid in the chicken manure composting process for guiding the composting production.

At present, the characterization of humic acid characteristics and the variation of humic acid in the composting process are mainly based on spectral information such as Ultraviolet-visible spectroscopy (UV-Vis), Infrared spectroscopy (IR), Fluorescence spectroscopy (FS) and $^{13}$C-nuclear magnetic resonance spectroscopy ($^{13}$C-NMR). These methods have gradually become an important means to study the transformation of organic matter [7, 8]. Although the spectra of humic acid are very important for the studies on the properties of humic acid, the analysis of humic acid is limited to a certain extent most because the spectroscopic studies require more rigorous pretreatment of samples, or have higher purity or solubility [9, 10]. Hence, further studies on the thermal properties and element composition of humic acid in composting process need to be carried out.

The aim of this paper was that under the condition of simulated composting in laboratory, the changes of humic acid quantity, element composition, spectral characteristics, and thermal properties during composting of chicken manure were systematically discussed, and the structural characteristics and activity of humic acid were judged accordingly, which provided a scientific basis for improving the reliability of composting quality and maturity evaluation of chicken manure and other agricultural wastes.

**Materials and Methods**

**Instruments and Reagents**

Vario-EL-III element analyzer (Germany Elementar elemental analysis Systems); DTG-60-differential thermal analyzer (Japan’s Shimadzu); Nicolet-5DXFT-Infrared spectrometer (American Thermo Nicolet Corporation); Lab-Tech UV-2000 UV-Visible spectrophotometer (American Lab-Tech company); Composting equipment: BIOTECH-30SS solid fermentation tank (Shanghai Baoxing Biological Engineering Co., Ltd.).

All reagents such as $K_2Cr_2O_7$ (AR), $FeSO_4$ (AR), $Na_3P_2O_7$ (AR), $HCl$ (AR), $H_2SO_4$ (AR), $NaOH$ (AR) and KBr (SP) were produced by the Beijing chemical factory.

**Composting Materials and Composting Scheme**

Chicken manure was taken from the hennery located in Shilipu Village, Changchun, Jilin province, China. Cornstalk was taken from the experimental field located in the Experimental Station of Jilin Agricultural University, Changchun, Jilin province, China. In order to adjust the ratio of carbon to nitrogen and maintain good ventilation, cornstalk was crushed to small pieces of about 2 cm straw and mixed with chicken manure. The composition and main components of composting materials are shown in Table 1.
Mixing chicken manure and cornstalk by the ratio of C/N = 23.8, and controlling the initial moisture content of 60%. Mandatory ventilation was used to regulate the ventilation volume so that the oxygen concentration in the exhaust gas was not less than 10%. The composting process was judged according to the change of the temperature of the compost: the composting temperature reached 50ºC in 3 days, the highest temperature reaches 61ºC in 5 days, and then gradually fell back and was lower than 50ºC in 13 days. When the temperature was close to the ambient temperature in 30 days, it indicated that the composting process was over. Three replicates of composting treatment were prepared and samples were taken at different composting times of 0, 2, 5, 10, 15, 20 and 30 days, respectively.

Methods of Sample Processing and Determination

Extraction and Purification of Humic Acid

The compost sample was extracted by oscillation with a mixed solution of 0.1 mol L⁻¹ NaOH and 0.1 mol L⁻¹ Na₄P₂O₇ at 70ºC in water bath. The extract was adjusted to pH 1.0 by H₂SO₄ solution, and the precipitate obtained was the sample of humic acid. The detail was referenced to the literature [11].

Determination of the Relative Quantity of Humic Acid

The absolute content of organic carbon in the samples of humic acid and composting was determined by the potassium dichromate volumetric method. The ratio of the former to the latter is the relative quantity of humic acid [11].

Elemental Analysis

Weighing HA samples 2 mg, wrapped in tin-boat and put into the elemental analyzer, analyzing the element contents of C, H, N and O under 850ºC.

Tonal Coefficient (ΔlgK) Analysis

Weighing 1.0 mg HA sample and dissolved into 10 mL 0.05 mol·L⁻¹ NaHCO₃ solution, measuring absorbance at the wavelength of 400 nm and 600 nm, respectively.

FTIR Spectrum Analysis

Finely grinding and mixing the HA samples with KBr according to the ratio of 1:100, placing in a dryer containing P₂O₅ for 24 hours, pressing into tablets under 10 MPa, measured by FTIR spectrometer scan under the wavelength ranging from 4000 cm⁻¹ to 350 cm⁻¹.

Differential Thermal Analysis

Weighing HA samples 2.0 mg, put in a special aluminum pool with a differential thermal analyzer, heating up at the rate of 5ºC·min⁻¹ up to 600ºC and keeping 30 minutes. Recording the weight loss and heat release the temperature range from 120ºC to 600ºC. Since the infrared spectra of the samples at the heating stage from room temperature to 120ºC was greatly affected by the moisture content of the sample, the data at the heating stage were not recorded.

Data Processing Method

The data were sorted out by Excel 2003 and analyzed by DPSv7.05 statistical software.

Results and Discussion

Variation of the Relative Quantity of Humic Acid in Chicken Manure Composting

The humification of organic materials can be caused by composting, and the relative amount of humic acid (HA(%) = (organic carbon content in HA/organic carbon content in compost) × 100%) is an important factor affecting the quality of humus. Fig. 1 shows the dynamic change rule of the relative amount of HA in the process of chicken manure composting. It can be seen from Fig. 1 that the relative amount of HA fluctuates obviously in the whole process of chicken manure composting. The general trend was to increase rapidly at the initial stage of composting, then decreased, and finally increased slowly again. The turning point was the 5th and 10th days of composting. After chicken manure composting, the relative amount of HA increased from 5.46% at the beginning of composting to 8.36%.

In the process of chicken manure composting, there was not only the formation of humic acid but also the transformation of humic acid to fulvic acid (i.e., the decomposition of humin). These two processes were carried out simultaneously in composting, and
they grew and fell with each other, therefore, they fluctuated greatly in the whole composting of chicken manure. At the initial stage of composting, the amount of HA increased rapidly, reaching the peak value at 5 days, which indicated that the formation speed of HA in the initial stage of composting was faster than the decomposition speed. At the same time, the fast decomposition of easily degradable organic matters (such as sugar, starch, etc.) in composting materials led to the ‘relative concentration’ of humic acid [12], which also caused the rapid increase of the relative amount of humic acid [13]. Chicken manure composting made HA increase by 52.7%, it indicated that composting treatment was beneficial to the formation and accumulation of humic acid, while it was also beneficial to the improvement of soil structure by fertilizing [14].

Changes of Element Composition of Humic Acid in Chicken Manure Composting

In general, the ratios of H/C, N/C, and O/C are the indicators of condensation and oxidation of humic substances. Table 2 shows the content of C, H, N and O. The ratios of H/C, N/C and O/C elements in chicken manure at different composting times also shown in Table 2. It indicated that in the process of chicken manure composting, the ratio of H/C of HA had a downward trend, from the initial 1.73 to 1.61. The result showed that the composting treatment was conducive to improving the condensation degree of HA molecules [15]. The changing trend of ratio of N/C of HA decreased at first, and then increased gradually, and there was a valley value of 0.07 in the middle stage of composting, which was probably caused by the rapid transformation of a large amount of nitrogen into ammonia gas in the early stage of composting, resulting in the faster degradation of nitrogen than carbon and in the later stage of composting, the change of nitrogen was relatively stable, the degradation of carbon was faster [16], nitrogen functional groups increased, and the ratio of N/C gradual increased. Due to the loss of C and O in different composting stages, there was no obvious regularity for the ratio of O/C.

| Time(d) | C (g·kg⁻¹) | H (g·kg⁻¹) | N (g·kg⁻¹) | O (g·kg⁻¹) | H/C | N/C | O/C |
|---------|------------|------------|------------|------------|-----|-----|-----|
| 0       | 502.5      | 72.50      | 69.28      | 355.7      | 1.73| 0.12| 0.53|
| 2       | 625.3      | 88.86      | 74.51      | 211.3      | 1.71| 0.10| 0.25|
| 5       | 605.1      | 83.31      | 58.53      | 253.1      | 1.65| 0.08| 0.31|
| 10      | 614.4      | 83.31      | 58.53      | 253.1      | 1.65| 0.08| 0.31|
| 15      | 542.0      | 73.69      | 44.16      | 340.2      | 1.63| 0.07| 0.47|
| 20      | 546.1      | 74.57      | 46.39      | 332.9      | 1.64| 0.07| 0.46|
| 30      | 568.9      | 76.46      | 66.71      | 287.9      | 1.61| 0.10| 0.38|

Note: H/C, N/C, O/C are mol ratios.
process, and finally decreased to 0.85, it indicated that the composting treatment promoted the degradation of small molecular proteins and carbohydrates with small molecular weight, simple structure and low aromatization level in HA [19]. The newly formed HA was being of the increased condensation degree, complex molecular structure, large molecular weight, and HA were more mature [20]. However, compared with the ΔlgK of HA in the normal cultivated soil with higher maturity, the value of ΔlgK is still higher [21, 22], indicating that HA in the composting chicken manure had the potential for further stabilization even in the late stage of composting, which was consistent with the conclusion of Romeela et al. [23]. It also showed that the activity of humus in compost was strong, and the relative molecular weight could be increased further, the compost may play an important role in the regeneration and activation of soil humus.

**Characteristics of the Infrared Spectrum**

Fig. 3 shows the infrared spectrum of humic acid in chicken manure at different composting time. The wider absorption peak at 3400 cm⁻¹ is from the stretching vibration of –OH belong to phenol and alcohol and C-H located in unsaturated carbon. The absorption peaks at 2920 cm⁻¹ and 2850 cm⁻¹ from the stretching vibration of C-H belong to hydrocarbon and aldehyde respectively. The absorption peaks at 1720 cm⁻¹ from the stretching vibration of C=O belong to carbonyl and carboxyl. The absorption peaks at 1620 cm⁻¹ from the stretching vibration of C=C belong to benzene ring or aromatic heterocycle in HA molecule. In 1050 cm⁻¹, there is an absorption peak related to the stretching vibration of the Si-O bond of silicate impurity, which may be related to the ash content in the sample [11]. During 0-30 days of the whole composting process, the absorption peaks of 3400 cm⁻¹ decreased gradually, and the absorption peaks of 2920 cm⁻¹ and 2850 cm⁻¹ of C-H stretching vibration of hydrocarbon and aldehyde were also gradually weakened, while the absorption peaks of 1620 cm⁻¹ of stretching vibration C=C belong to benzene ring or aromatic heterocycle is gradually strengthened. Meanwhile, the absorption peaks of 1720 cm⁻¹ of C=O stretching vibration of carbonyl and carboxyl are also gradually enhanced and combined with that of 1620 cm⁻¹. In addition, the ratio of absorption peak areas of 2920 cm⁻¹ and 2850 cm⁻¹ to that of 1620 cm⁻¹ decreased significantly with the increase of composting time (Table 3). The above results showed that the proportion of carbonyl, carbonyl and aromatic structure of HA molecules in chicken manure was higher and higher, while the content of -OH and aliphatic C-H was lower and lower with the increase of composting time [24, 25]. It was further proved that composting treatment was beneficial to the decomposition of simple compounds and the formation of complex organic matters, and promoted the aromatic degree of HA molecular structure in chicken manure [24].

**Change of Thermal Property of Humic Acid**

Differential thermal analysis (DTA) is a technology to measure the temperature difference between the target substance and the reference substance according to the change of temperature under the programmed temperature control. The heat release and heat weight loss of HA in chicken manure composting were studied by DTA in order to clarify the structural change characteristics of HA in the process of chicken manure composting. Fig. 4 shows the effect of different composting time on the thermal properties of HA in chicken manure. It indicated that there were two
exothermic processes of medium temperature and high temperature respectively between 250-370°C and 410-540°C. The exothermic peak of medium temperature is caused probably by the cracking of the peripheral materials of the aromatic nucleus of HA, which represented the exothermic reactions such as the decomposition of the aliphatic compounds in the humic molecules and the decarboxylation of the peripheral functional groups. The exothermic peak of high temperature was generally relatively independent, which was caused by the complete oxidation of humic substances and the decomposition of the aromatic compounds in the molecules [26]. In terms of peak temperature, with the extension of composting time, both of medium temperature exothermic peak and high temperature exothermic peak increased, which shows that the more complex the molecular structure is, the higher the temperature is required when the chemical bond breaks, i.e., the lower the degree of freedom of HA molecule.

The exothermic reaction is accompanied by weight loss. The thermogravimetric analysis of all HA samples shows that there was obvious weight loss at the middle-temperature exothermic peak and high-temperature exothermic peak (Table 4), indicating that the content of fatty chain structure and aromatic ring structure in HA molecule was rich relatively. With the process of composting, the weight loss at the exothermic peak of high temperature showed a trend of increasing gradually, i.e. the proportion of aromatic structure increased.

**Table 3. Effect of different composting time on main absorption peak area of infrared spectrum of HA.**

| Time (d) | 2920 (cm⁻¹) | 2850 (cm⁻¹) | 1620 (cm⁻¹) | (2920+2850) (cm⁻¹)/1620 (cm⁻¹) |
|---------|-------------|-------------|-------------|---------------------------------|
| 0       | 3.42        | 0.41        | 7.45        | 0.51                            |
| 2       | 2.37        | 0.57        | 8.22        | 0.36                            |
| 5       | 2.79        | 0.34        | 10.11       | 0.31                            |
| 10      | 3.01        | 0.3         | 14.52       | 0.23                            |
| 15      | 2.36        | 0.46        | 14.41       | 0.20                            |
| 20      | 1.64        | 0.72        | 11.4        | 0.21                            |
| 30      | 1.47        | 1.04        | 18.22       | 0.14                            |

**Table 4. Effect of different composting time on the weight loss of HA in chicken manure.**

| Time (d) | Medium temperature | High temperature |
|---------|---------------------|------------------|
|         | Peak temperature (°C) | Range (°C) | Loss (mg·g⁻¹) | Peak temperature (°C) | Range(°C) | Loss (mg·g⁻¹) |
| 0       | 296                 | 248-359       | 327.9        | 475                 | 411-513   | 164.8        |
| 2       | 297                 | 249-360       | 417.4        | 484                 | 420-522   | 211.3        |
| 5       | 299                 | 251-362       | 399.8        | 490                 | 426-528   | 297.9        |
| 10      | 300                 | 252-363       | 346.6        | 491                 | 427-529   | 288.6        |
| 15      | 303                 | 255-366       | 415.2        | 490                 | 426-528   | 323.4        |
| 20      | 306                 | 258-369       | 407.8        | 496                 | 432-534   | 336.3        |
| 30      | 307                 | 259-370       | 401.9        | 501                 | 437-539   | 326.7        |
Conclusions

(1) The composting process was conducive to the formation of humic acids based on the increase of the relative amount of HA from 5.46% to 8.36% in the composting process of chicken manure.

(2) The change of humic acid element composition showed that the degree of molecular condensation increased.

(3) The decrease of color coefficient (ΔlgK) showed that the proportion of aliphatic structure of HA reduced and the proportion of aromatic structure increased in the process of composting, composting treatment was beneficial to the decomposition of simple compounds and the formation of complex organics, and the HA component tended to mature in chicken manure. Furthermore, the molecular structure of humic acid in chicken manure composting still had the potential to stabilize after fertilization in soils.

(4) Both the exothermic peak temperature and weight loss increased with the extension of composting time, indicating that the molecular structure of HA became more complex in composting chicken manure.

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Conflict of Interest

The authors declare no conflict of interest.

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