STUDY ON DIFFERENTIAL PERFORMANCE OF MICRO-NANO HUMIC ACID FOR HEAVY METALS IN SOIL POLLUTED BY LIVESTOCK MANURE

SUN QIAN

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STUDY ON DIFFERENTIAL PERFORMANCE OF MICRO-NANO HUMIC ACID FOR HEAVY METALS IN SOIL POLLUTED BY LIVESTOCK MANURE

by

SUN QIAN

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LIST OF SYMBOLS

mol	Mole
mm	Millimetre
℃	Degree Celsius
%	Percentage
nm	Nanometre
mL	Millilitre
L	Litre
g	Gram
cm^{-1}	Wavelength
m	Meter
mg	Millimeter
T	Temperature
t	Time
kg	Kilogram
min	Minutes
V	Volume
h	Hour
µg	Microgram
d	Day
hm^2	Hectare
µm	Micron
rpm	Round per minute

LIST OF ABBREVIATIONS

pH	Concentration of hydrogen ions
H_2O	Water
Zn	Zinc
K	Potassium
Mg	Magnesium
C	Cement
CH	Hydrogen-carbon link
Ca	Calcium
Cu	Copper
NH_4^+-N	Ammonium nitrogen
SEM	Scanning electron microscopy
TEM	Transmission Electron Microscopy
USM	Universiti Sains Malaysia
SPSS	Statistical Package for the Social Sciences
HCl	Hydrochloric acid
N	Nitrogen
O-H	Hydroxide
P	Phosphorus
K	Potassium
Cl	Chloride
C=O	Carbonyl group
C-O-O	Carboxylate
COOH	Carboxylic acid
EDTA	Ethylenediamine tetraacetic acid
FTIR	Fourier transform infrared spectroscopy
3D-EEM	3D excitation-emission matrix
NMR	Nuclear magnetic resonance
NaOH	Sodium hydroxide
SO_4^{2-}	Sulfate Standard
$Na_2P_4O_7$	Sodium tetraphosphate
Humic acid	HA

EDS	Electron dispersion spectroscopy
XPS	X-ray photoelectron spectroscopy
FA	Fulvic acid
HS	Humin
SDS	Sodium dodecyl sulfate
PAM	Polyacrylamide
SBR	Sequencing batch reactor
UASB	Upflow anaerobic sludge
Pb	Lead
As	Arsenic
Co^{2+}	Cobalt(II)
Cr	Chromium
C-O	Carbon oxygen bond
CTAB	hexadecyl trimethyl ammonium bromide
DTPA	Diethylenetriaminepentaacetic acid
CH_3COOH	Acetic Acid
NH_4NO_3	Ammonium Nitrate
NH_4F	Ammonium fluoride
HNO_3	Nitric acid
NHA	Nano humic acid

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KAJIAN PRESTASI PEMBEZAAN ASID HUMIK MIKRO-NANO UNTUK LOGAM BERAT DI TANAH YANG TERCEMAR OLEH TINJA TERNAKAN

ABSTRAK

Pemulihan tanah tercemar logam berat telah menjadi titik panas dan sukar dalam penyelidikan akademik. Kesan berbahaya logam berat pada organisma secara langsung berkaitan dengan kandungan berkesan. Oleh itu, industri bercadang untuk mengurangkan kandungan berkesan logam berat dalam persekitaran tercemar dengan menambah bahan penjerap, iaitu teknologi pempasifan in-situ. Asid humik mikro-nano mempunyai kawasan permukaan khusus yang lebih besar dan aktiviti permukaan yang lebih kuat, dan struktur liang unik dan ciri-ciri morfologinya menguatkan lagi kelebihan kawasan permukaan tertentu dan mengoptimumkan proses penjerapan. Pada skala nanometer, kesan saiz kuantum mungkin memberikan keupayaan penjerapan kimia khas. Selepas 30 hari, penjerapan asid humik dengan dos 250 mg/L mengurangkan kandungan kuprum dalam baja daripada 31.212 mg/L kepada takat terendah 7.348 μg/L. Asid nano-humik boleh mengurangkan kandungan kuprum dalam baja ternakan kepada 6.28 μg/L, dan boleh menyerap 79.9% kuprum. Selepas 60 hari, Penjerapan asid humik nano mengurangkan kandungan kuprum dalam kumbahan najis daripada 31.212 μg/L kepada 9.203 μg/L, dan kadar penjerapan boleh mencapai 70.5%. Dalam pertimbangan keseluruhan, jumlah penggunaan optimum asid nano-humic dalam tanah ialah 25 mg/kg. Asas teori yang disediakan oleh kajian ini menawarkan sumbangan berharga kepada pembangunan teknologi pemulihan tanah tercemar logam berat yang praktikal, cekap dan selamat.

STUDY ON DIFFERENTIAL PERFORMANCE OF MICRO-NANO HUMIC ACID FOR HEAVY METALS IN SOIL POLLUTED BY LIVESTOCK MANURE

ABSTRACT

Remediation of heavy metal contaminated soil has become a hot and difficult point in academic research. The harmful effects of heavy metals on organisms are directly related to the effective content. Therefore, the industry proposes to reduce the effective content of heavy metals in polluted environment by adding adsorbents, that is, in-situ passivation technology. Micro-nano humic acid has larger specific surface area and stronger surface activity, and its unique pore structure and morphological characteristics further amplify the advantages of specific surface area and optimize the adsorption process. At the nanometer scale, the quantum size effect may give it special chemical adsorption ability. After 30 days, the adsorption of humic acid with a dosage of 250 mg/L reduced the copper content in manure from 31.212 mg/L to the lowest point of 7.348 μg/L. Nano-humic acid can reduce the copper content in livestock manure to 6.28 μg/L, and can adsorb 79.9% copper. After 60 days, The adsorption of nano humic acid reduced the copper content in fecal sewage from 31.212 μg/L to 9.203 μg/L, and the adsorption rate could reach 70.5%. In overall consideration, the optimal application amount of nano-humic acid in soil is 25 mg/kg. This topic examines the adsorption mechanism and adsorption differences of heavy metal ions in livestock and poultry manure pollution by micro-nano graded humic acid. The theoretical basis provided by this study offers a valuable contribution to the development of practical, efficient and safe heavy metal contaminated soil remediation technology.

CHAPTER 1 INTRODUCTION

1.1 Background of Study

In recent years, the breeding industry has undergone a gradual transition from the traditional free-range breeding model to large-scale and intensive development. The discharge of untreated livestock and poultry manure has resulted in an excessive pollution load pressure on the land in the area (Tang, 2016; Su, 2006; Xu et al., 2004; Liao et al., 2013). The pollution of heavy metals to soil is diverse. When heavy metals exist in the soil in this area for a long time, the soil quality in this area will be reduced (Wu et al., 1994; Wang, 1997). Because of the high content of heavy metals, it will affect the normal growth of crops, and it will affect the healthy growth of livestock when they are exposed to heavy metals (Yang et al., 2000; Gao et al., 2006). At present, the pollution of heavy metals in soil is serious. If heavy metals invade the surface and enter the waters, it will affect the global water environment (Xue et al., 2002; Qu et al., 2005), cause serious waste of ecological resources and destroy the balance of natural ecosystems (Wang et al., 2006; Zhang et al., 2012; Zhu et al., 2008; Chen et al., 2004). Heavy metal soil is harmful to human health. Volatile heavy metal elements in polluted soil usually pass through the respiratory tract and affect people's metabolic function, thus causing a series of diseases.

The traditional engineering measures of soil contaminated by heavy metals mainly include soil replacement and deep ploughing. Deep ploughing and ploughing are used for lightly polluted soil, while exotic soil and soil replacement are common methods for heavily polluted areas. Engineering measures are classic measures to control heavy metal pollution in soil, which have the advantages of thoroughness and stability. However, the implementation of engineering measures is large, the investment cost is high, the soil structure is destroyed, and the soil fertility is reduced.

In addition, the exchanged contaminated soil should be piled up or treated (Sun et al., 2015).

In recent years, chemical remediation technology of heavy metal contaminated soil, especially in-situ passivation remediation technology, has been paid more and more attention because of its advantages of high efficiency, rapidity and low cost (Yang et al., 2011; Li et al., 2005). In-situ passivation technology can improve the utilization rate of soil resources to some extent. In the process of treating heavy metal contaminated soil by in-situ passivation technology, through a series of redox reactions, it is beneficial to change the form of heavy metal contaminated soil, thus effectively reducing the heavy metal content of heavy metal contaminated soil (He, 2004; Wu et al., 2007; Wang et al., 2004; Fan et al., 2006). However, some in-situ passive adsorbents are easy to cause secondary pollution in the process of remediation of contaminated soil. Therefore, when using adsorbents for soil remediation and purification in areas with high heavy metal content in soil, we must constantly seek excellent adsorption methods to avoid damage to soil structure, ensure soil quality, and at the same time use adsorbents to adsorb heavy metal elements in soil, thus reducing pollution to groundwater and soil, slowing down heavy metal elements in soil, maintaining the best state of soil and enhancing soil remediation ability.

Humic acid is a naturally occurring substance that is widely distributed in various geological formations, including lignite, peat, and weathered coal (Tong et al., 2021; Chen et al., 2010). It is a biomass raw material with considerable reserves and considerable potential for further development. The utilisation of humic acid as a raw material for the preparation of soil heavy metal adsorbents has been

demonstrated to not only effectively remediate heavy metal pollution (Sposito, 1986; Liu et al., 1992; Niu et al., 2008), but also to enhance the physical, chemical and biological properties of soil, thereby offering significant potential for application, Micro-nano technology can improve the adsorption performance of humic acid. Micro-nano humic acid is prepared by settling shear technology, and the adsorption capacity of modified micro-nano humic acid is increased, which can improve the remediation efficiency of humic acid in soil heavy metals.

The adsorption performance of heavy metal adsorbents is largely determined by their distinctive surface structural characteristics and surface chemical properties (Lou et al., 2004; Bai et al., 2000). Micro-nanonisation of humic acid is a common method of enhancing the adsorption performance of heavy metals in soil. This topic examines the adsorption mechanism and adsorption differences of heavy metal ions in livestock and poultry manure pollution by micro-nano graded humic acid. In this study, micro-nano adsorption experiments were also carried out to study the difference of adsorption capacity of micro-nano humic acid and ordinary humic acid for heavy metals in soil and manure contaminated by livestock manure. The theoretical basis provided by this study offers a valuable contribution to the development of practical, efficient and safe heavy metal contaminated soil remediation technology. Furthermore, the study has important theoretical and practical significance for the further advancement of livestock and poultry manure soil pollution control.

1.2 Research problem

The discharge of untreated livestock and poultry manure has resulted in an excessive pollution load pressure on the land in the area. Humic acid molecules have

many functional groups, which can adsorb heavy metal ions. Micronized and nanocrystallized humic acid changes its molecular structure and has better adsorption characteristics. Micro-nano technology can improve the adsorption performance of humic acid and influence the remediation efficiency of humic acid in soil heavy metals.

Silvia (2004) studied the molecular structure of humic acid, and analysis showed that the active groups within the humic acid molecule are of great significance to the passivation effect of heavy metals. In fact, humic acid itself is an amorphous organic polymer compound, which contains active groups such as carboxyl, phenolic hydroxyl, quinone, carbonyl, alcoholic hydroxyl, and methoxy groups, making humic acid molecules have the following characteristics for metal ions: exchange performance, dispersion performance, cohesion performance, complexing performance, buffering capacity, adsorption capacity, etc (Szabó et al., 2004; Fernández-Escobar et al., 1996; Chen et al., 2004).

However, humic acid has a cyclic structure of macromolecules. At the same time, humic acid with high molecular weight has low water solubility, high adsorption free energy, can generate a large electrostatic field, and has poor mobility. Therefore, humic acid can be micronized and molecular weight graded to increase the activity of functional groups, thereby improving its adsorption performance.

1.3 Research questions

i How does livestock manure cause heavy metal pollution in soil?

ii How to obtain nanoscale humic acid using alkali acid precipitation and high shear technology?

iii How does micro-nano humic acid absorb heavy metals in contaminated soil?

iv How does nanoscale humic acid adsorb heavy metals in livestock manure?

1.4 Research objectives

i To evaluate the changes in the physical structure and chemical components of humic acid after micro-nano-graded humic acid

ii To determine the relationship between the use of humic substances and soil environment

iii To characterise the adsorption of heavy metals in manure and fecal sewage by nano-humic acid

iv To assess the influencing factors of Micro-nano humic acid on the adsorption performance of heavy metals heavy metals from soil contaminated by livestock

1.5 Scope of study

This project intends to proceed from the perspective of nanophysical conditions, and plans to conduct research from the following aspects: (1) Preparation of micro-nano humic acid and its structural characterization; (2) Study on the influence of humus on soil environment; (3) Micron humic acid adsorbs heavy metals such as Cu and Zn in soil; (4) Adsorption of heavy metals in livestock and poultry manure by nano-humic acid; (5) Nano-humic acid adsorbs heavy metals from soil contaminated by livestock and poultry waste; (6) The effect of nano-humic acid combined with hydroponic nutrient solution on the growth quality of wheatgrass.

In this study, micro-nano humic acid was extracted from lignite and livestock waste by high-speed shearing technology. Because the different particle sizes of micro-nano particles caused different physical and chemical properties, the adsorption performance of heavy metals in soil was different. Micro-nano humic acid as a heavy metal passivator provided theoretical basis for the remediation of heavy metals in livestock waste. It provides theoretical reference and practical basis for the comprehensive utilization of livestock waste and humic acid, improving soil environment and promoting the development of modern agriculture.

1.6 Theoretical contribution

Humic acid is modified by nanotechnology, and this technology can make humic acid have specific physical and chemical properties, thus optimizing and improving the adsorption performance of humic acid materials. Nano-humic acid is a new type of soil in-situ passivation material with strong adsorption and colloidal stability, which has developed rapidly in recent years. This technology can make humic acid have specific physical and chemical properties, thus optimizing and improving the adsorption performance of humic acid materials. Nano-humic acid is a new type of soil in-situ passivation material with strong adsorption and colloidal stability, which has developed rapidly in recent years. The research of Cheng Liang and others shows that reducing the particle size of humic acid can effectively improve the surface energy, surface area, swelling property, colloidal stability and surface active sites of humic acid (Cheng et al., 2016). Combined with molecular weight classification, its internal structure is changed to improve adsorption characteristics and adsorption stability. Therefore, we should comprehensively grasp

it from the perspectives of physics and chemistry in order to break through the bottleneck of humic acid technology application.

1.7 Practical contribution

There is a lack of research on the treatment of soil contaminated by livestock manure based on nano-materials. In this study, micro-nano humic acid was used as the repair material. The active groups in its structure can be used to adsorb heavy metals, and the ability of complexing and chelating can be used to passivate heavy metals in soil. Studying the remediation of heavy metal pollution in soil provides a new technology for soil pollution control and a new development space for the application of micro-nano materials in regional environmental pollution control. Up to now, there is a lack of research on the treatment of soil contaminated by livestock manure based on micro-nano materials. It provides a theoretical basis for the development of practical, efficient and safe remediation technology for soil contaminated by heavy metals, and has important economic significance and social benefits for promoting the in-depth development of soil pollution control by livestock manure.

CHAPTER 2 LITERATURE REVIEW

2.1 Definition of Humus

Humus is an organic polymer formed and accumulated by animals and plants as showen in Figure 2.1, especially plants, through microbial decomposition and transformation and a series of biogeochemical processes (He, 2003). It is the most widely distributed natural organic substance in the ecological environment of the earth. Under certain conditions, it is a charged organic colloid, which contains many functional groups, such as carboxyl groups and phenolic hydroxyl groups, and has high reactivity (Li, 2003; Lu et al., 2000; Geyder et al., 2000; Chai et al., 2008). It can improve the physical and chemical properties of soil, stimulate the growth of crops, strengthen the stress resistance of crops and improve the quality of agricultural products (Mao et al., 2007; Fong et al., 2007; Gu et al., 2000). Therefore, humus has been widely concerned in agricultural production, and various humic acid fertilizers have widely appeared in the market.

Humus is a polymer compound formed by condensation of aromatic compounds (polyphenol type) and nitrogen-containing compounds (polypeptide type) with a small amount of reducing substances (polyuronic acid glycoside type) (Sun et al., 2015). Humus is a kind of organic compounds formed in soil, water and sediments after dead organisms decay. Because of their very complex properties, they can only be defined in a very negative sense, that is, they are organic compounds in soil, water and sediments except protein, polysaccharides and lipids, which account for most of the total organic matter in the environment, about 70%. Humus is a polymer compound formed by condensation of aromatic compounds (polyphenol type) and nitrogen-containing compounds (polypeptide type) with a small amount of reducing substances

(polyuronic acid glycoside type) (Klucakova et al., 2014; Cavusoglu et al., 2017; Martyniuk et al., 2003).

Figure 2.1 Humus in soil

After a long study, the definition of humus has been further improved. With the development of modern analytical technology, the structure of humus is becoming clearer. Humus is formed in several ways. Including glycosaminoglycan condensation theory, polyphenol theory, multi-enzyme theory originated from lignin, lignin theory, cell autolysis theory and microbial synthesis theory. All these pathways may play a role in soil, but they are affected by different environmental factors.

2.2 The formation of humus

Humus is a series of multi-component, complex, brown to black substances formed by secondary reactions (Li et al., 2003; Li et al., 2004), which is widely

distributed in the land and water environment and is the most important organic carbon pools in land and ocean (Aiken et al., 1985; Schnitzer, 1991). Humus includes soil humus, water humus, wetland humus, geological humus and artificial humus (Wang et al., 2002; Wang et al., 2011; Shahbazi et al., 2019). Although the basic components of humus are similar, there are many kinds of precursors and reaction types involved in the formation of humus, so there may be many ways of combination between intermediates, which leads to the diversity of humus macromolecules in chemical form. We can also think that the difference between humic acid and fulvic acid is only their molecular weight and degree of polymerization. In fact, humus can be formed through the above-mentioned mechanisms, and they can be transformed into the starting materials.

The earliest study of humus can be traced back to the late 18th century. In 1786 Achard and in 1797 Vaquelin treated the soil with acid and extracted with alkali, respectively. A black amorphous precipitate was obtained when the extract was acidified. In 1804, Saussure first introduced the word "humus" (Latin "soil") to describe the dark organic matter in soil, and determined that it contained more C than the original organic matter, but more H and O (Zara et al., 2017; Stefanova et al., 2014; Vrantsi et al., 2021; Sachs et al., 2010). In 1809, the establishment of soil science was due to the formation of humus theory. By the end of the 19th century, it was suggested that humus is a complex organic mixture, mainly colloidal substances, which are weakly acidic. The researchers have reclassified humus and determined its chemical properties and structure. From 1950s to 1960s, with the development of analytical technology, people began to pay attention to the specific structure of humus and find out its microscopic characteristics, and the mystery of humus was gradually uncovered. Figure 2.2 shows the formation process of humic substances, there are several ways to

form humus: sugar amine condensation theory, polyphenol theory, lignin-derived polyphenol theory, lignin theory, cell autolysis theory and microbial synthesis theory are arranged in sequence (Zhao, 2010; Tanaka, 2012; Li et al, 2011; Shi, 2016; Senesi et al., 1996; Zhang, 2016; Chen et al., 2010; Kulikowska et al, 2015).

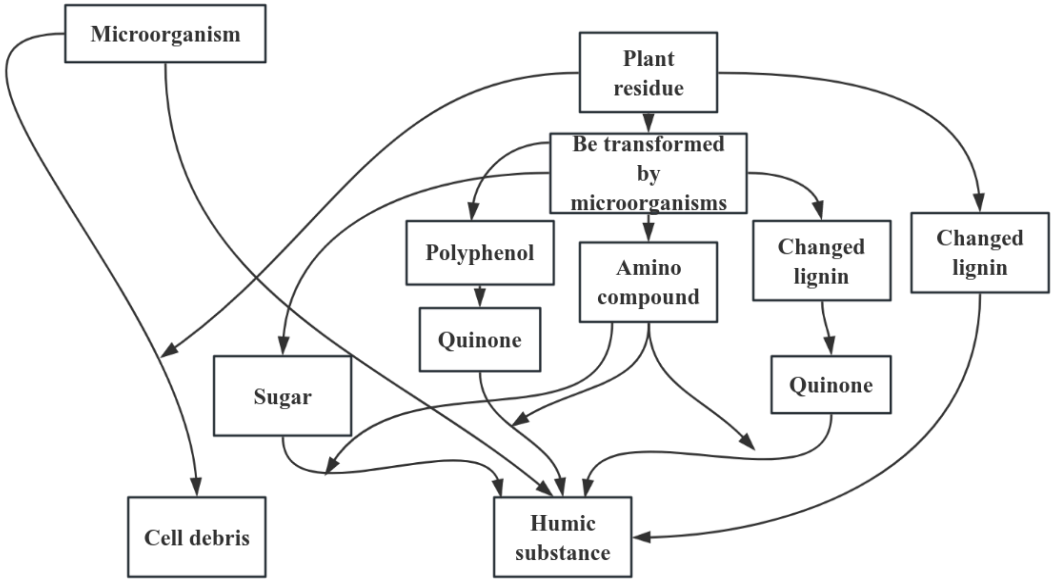

Figure 2.2 Formation process of humic substances (Huang., 2006)

Although there is not a completely satisfactory mechanism to explain the formation pathway of humus, most scholars tend to adopt the polyphenol theory, and Stevenson put forward the formation pathway of humus in 1994. According to this theory, some low-molecular-weight organic substances are oxidized, condensed and polymerized by microorganisms to generate humus polymers. According to this theory, the main component of humus are polyphenols derived from lignin.

2.2.1 Sugar amine polycondensation theory

According to this theory, humus is a brown N-containing polymer formed by non-enzymatic polymerization of reducing sugar and amino acids produced by microbial metabolism.

2.2.2 Polyphenol theory

Kononova (1966) thinks that plant materials are degraded into phenols and amino acids by microorganisms, and humus is formed by chemical oxidation and polymerization. The nature of plant tissue does not affect the kinds of humus produced in the end. Low-molecular-weight FA represents the first stage of humification, and FA is further concentrated to form HA and Hu (Huang, 2009).

2.2.3 Polyphenol theory originated from lignin

Route 2.2.3 is very similar to route 2.2.2. The difference between them is that lignin plays an important role in pathway 2.2.3 and must be converted into polyphenols first. In pathway 2.2.2, polyphenols are synthesized by microorganisms using non-lignin substrates (such as cellulose) as carbon source.

2.2.4 Lignin theory

It was put forward by Waksman (1936), who believed that the appearance of plant tissues resistant to microbial invasion, especially lignified tissues, changed more or less in soil, thus forming humus (Huang, 2009). Figure 2.3 shows the six basic structures of lignin, and the properties of plant components have great influence on the properties of final humus. HA with relatively large molecular weight represents the first stage of humus, which is decomposed into FA by microorganisms and finally mineralized into CO_2 and H_2O. However, according to the nature and size of primitive plant molecules, humus with relatively low molecular weight can be formed in the early stage of mineralization.

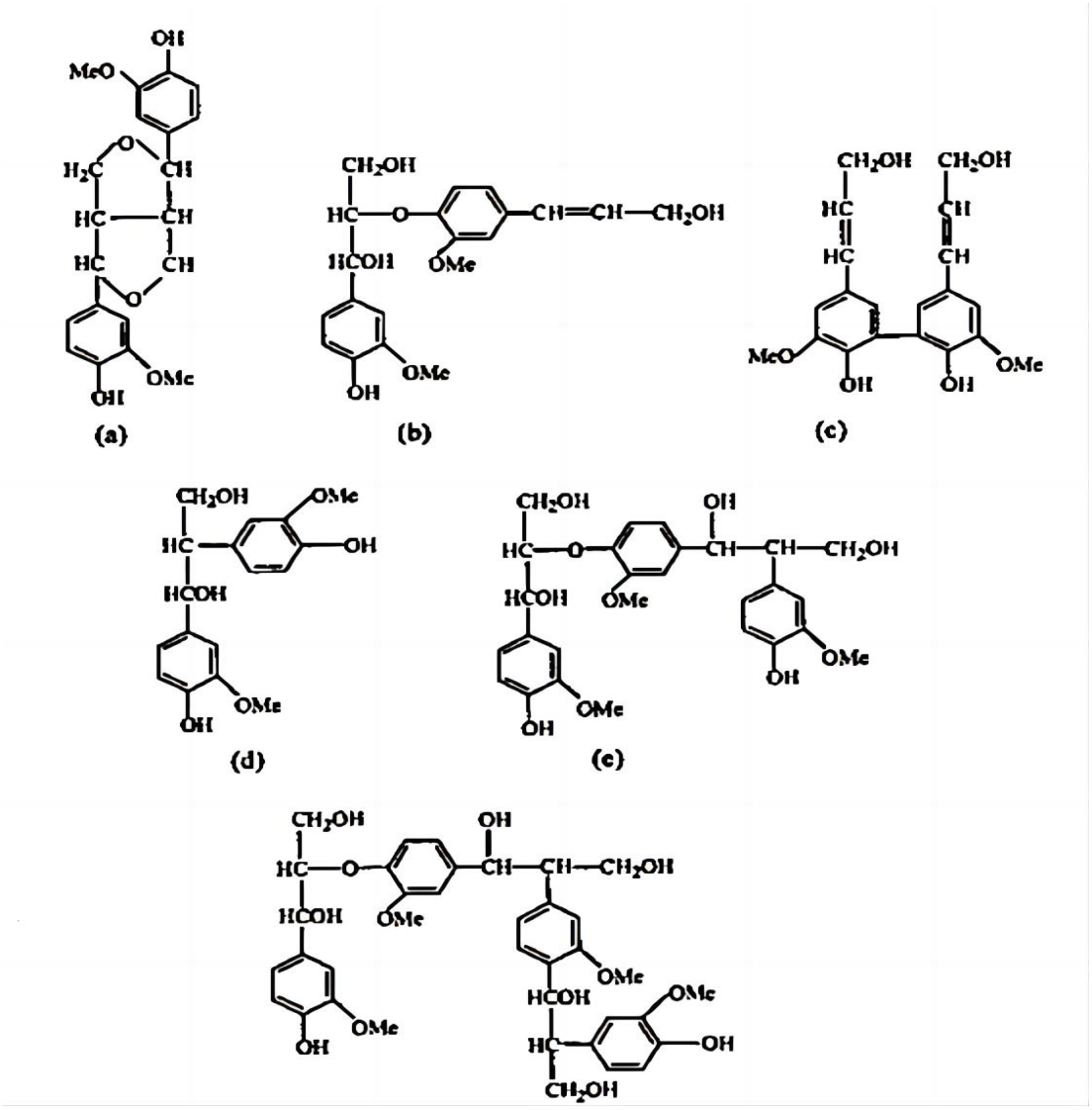

Figure 2.3 Six basic structures of lignin (Huang., 2006)

2.2.5 Autolysis theory of cells

According to this theory, humic substances are autolysis products of plants and microbial cells after their death, which are formed by random aggregation or polymerization of free radicals.

2.2.6 Theory of microbial synthesis

Microorganisms synthesize humic substances with relatively high molecular weight in cells, and release them into the soil when microorganisms die and cells

disintegrate. HA with relatively high molecular weight represents the first stage of humification, and then it is degraded into FA by extracellular microorganisms until it is finally mineralized.

These pathways may work in all soils, but their importance varies according to environmental conditions.

2.3 Separation and purification of humus

2.3.1 The separation of humus

Before studying humus in soil, it is necessary to extract humus from soil first, and then separate and purify it. At present, the composition and structure of soil humus are still inconclusive, and one of the main reasons is the extraction problem. When the chemical properties of the extractant are too strong, it will change the structure and properties of humus and destroy some non-humic substances, which will increase the difficulty of identification. When the chemical properties of the extractant are too weak, humus and minerals cannot be completely separated and the yield is too low. Some organic-mineral composite colloids, which are combined by molecular attraction and electrostatic attraction, are so firmly combined that they can't be completely separated even by acid hydrolysis with moderate concentration. Another problem is that it is not easy to distinguish humic substances from non-humic substances completely. At present, NaOH or NaOH+Na_4PO_7 is the most widely used, and its extraction rate is higher than other extractors (Table 2.1).

Table 2.1 Humus extraction and its function (Huang., 2006)

Extractant	Extract organic matter (%)
NaOH	80
Na_2CO_3	30
Na_4PO_7, NaF	30
Organic acid salt	30
Organic chelate, acetylacetone, 8-carbonyl quinoline, N-hexane, chloroform	30
HCOOH(+LiF, LiBr, HBF_4)	55
C_3H_6O-H_2O-HCl	20
NaOH+Na_4PO_7	90

According to its solubility in acid and alkali solutions, humus can usually be divided into three main components (Table 2.2): (1) Humic acid (HA), which is soluble in dilute alkali solution but flocculates in acidified alkali solution extract, that is, the component soluble in alkali but insoluble in acid; (2) Fulvic acid (FA) is the component that can still be dissolved in the liquid phase after acidification of dilute alkali extract, that is, both acid and alkali are soluble; (3) Humin (Hu), a humus component that can not be extracted from soil samples by dilute alkali and dilute acid, is a component that is neither soluble in acid nor alkali. Soluble fulvic acid components are usually small in molecular weight; The molecular weight of humic acid components that are not soluble is large; Most of the least soluble humin components are closely combined with soil minerals.

Table 2.2 Comparison of different grouping methods of humus (Huang et al., 2006)

Method for extracting humus	FA	HA	Residual part
2%HCl, 1%NaOH,	19	40	42
5%HCl 5%NaOH	33	48	19
0.1% Na_4PO_7 0.1%%NaOH,16h	18	29	53
0.1% Na_4PO_7	38	35	27

2.3.2 Purification of humus components

The separated humic acid (Figure 2.4) and fulvic acid should be purified to minimize non-humic substances. According to the method determined by the International Humic Acid Association (IHSS), the purification of humic acid is to suspend the crude humic acid precipitate in dilute HCl, add HF and shake to remove silicic acid, repeat this process twice, then dialyze and freeze-dry to obtain the pure humic acid; The purification of fulvic acid is to adsorb fulvic acid with macroporous adsorbent to remove protein and other non-humus parts, then elute fulvic acid with alkali, add HCl and HF for desilication, then elute with adsorbent, and finally freeze-dry to obtain pure fulvic acid.

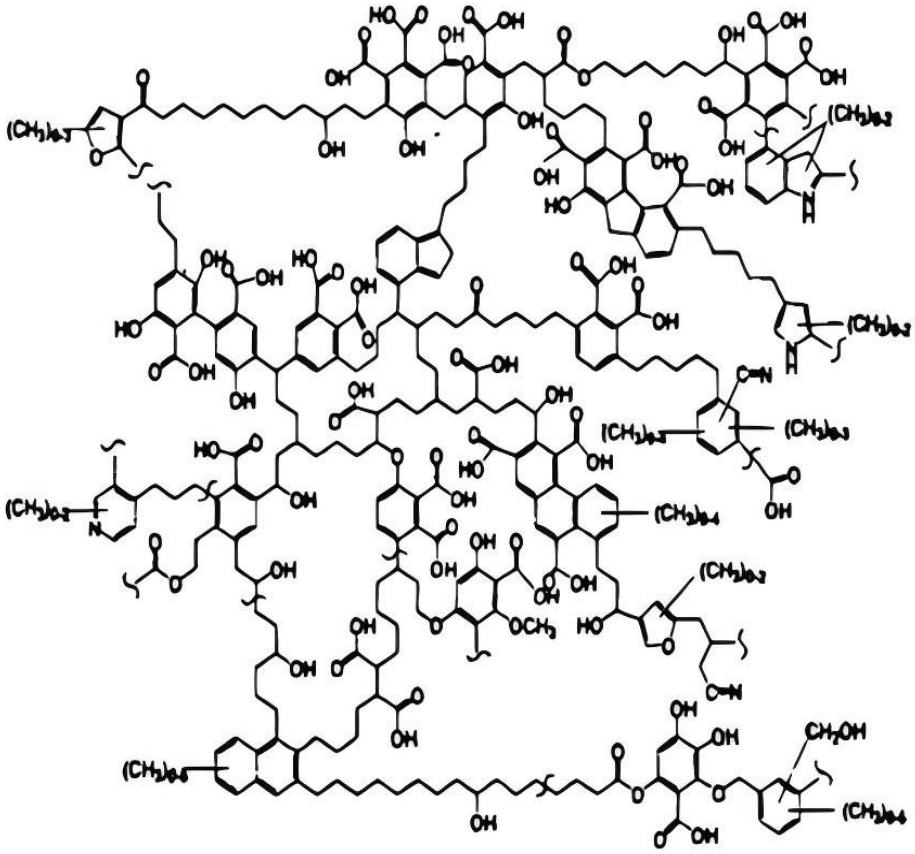

Figure 2.4 Stevenson structure model of HA (Li, 2012)

2.3.3 Separation and purification of humus

According to the solubility characteristics of humus at different pH values, humus can be divided into fulvic acid, humic acid and humic acid. Fulvic acid is a component of humus that is soluble in acid and alkali, humic acid is only soluble in acid but not alkali, and humin is insoluble in acid and alkali. Alkaline solvent is the earliest reagent used to extract humus. At present, NaOH and KOH are the most effective and widely used extractants, and the extraction concentrations include 0.1 mol/L, 0.5 mol/L and 1 mol/L. Other extraction agents include salt solutions such as sodium fluoride and sodium pyrophosphate, and organic solutions such as acetone.

2.3.4 Characterization technology of humus

The structure, composition and chemical properties of humus have been puzzling humus scholars at home and abroad, so scholars have been using various advanced technical means to analyze humus. The composting process is essentially the rapid stabilization and humification of organic matter in compost. The most appropriate and reliable method to analyze the operation and maturity of composting system is to analyze the chemical structure and functional characteristics of organic functional groups, and compare them with humus in natural soil (Sachs et al., 2010; Doskocil et al., 2014; Li et al., 2003).

Table 2.3 is a summary of the commonly used structural analysis methods of humic substances. According to different forms and principles, we can choose the appropriate analysis and testing methods. When the sample is solid, we can choose Fourier transform far infrared spectrum analysis, ^{13}C NMR analysis, Pyrolytic analysis and X-ray diffraction pattern analysis, and when the sample is liquid, we can choose Ultraviolet spectrum analysis, Fluorescence spectrum analysis, and Gel permeation chromatography (Yao et al., 2014). At present, the research on soil humus is in-depth, and mature methods and techniques for extracting and characterizing soil organic matter have been developed. As an important part of organic matter, humus accounts for 65% -80% of soil organic matter (Wang et al., 2010; Wu et al., 2021; Lamar et al., 2009). Humic matter in soil is mainly composed of carbon, hydrogen, oxygen, nitrogen, phosphorus and sulfur, and contains a small amount of ash elements such as calcium, magnesium, iron and silicon. The analysis of humus in composting systems can learn from the analysis methods of soil humus. At present, various analytical methods in soil have been effectively applied to the analysis and detection of humus (Baigorri et al., 2009; Wang et al., 2010; Schmeide et al., 2012).

Table 2.3 Common structural analysis methods of humic substances (Yao et al., 2014)

Analytical method	Principle	Sample morphology
Elemental analysis	The contents of carbon, oxygen, nitrogen, hydrogen and other elements were analyzed and determined	solid state
Ultraviolet spectrum analysis	Through the ultraviolet scanning spectrum of the sample, the functional groups on the surface of the substance were analyzed	liquid state
Fluorescence spectrum analysis	Through the fluorescence emission, excitation and scanning spectrum of the sample, the functional groups on the surface of the substance were analyzed	liquid state
Fourier transform far infrared spectrum analysis	The functional groups on the surface of humic acid were analyzed by infrared absorption spectrum of the sample	solid state
Gel permeation chromatography	Separation of polymer molecules or particles with different hydrodynamic volumes by chromatographic column filled with porous non-adsorption gel	liquid state
^{13}C NMR analysis	Carbon content of different components in humic acid was analyzed by ^{13}C NMR spectrum of samples.	solid state
Pyrolytic analysis	The material is pyrolyzed into low molecular products at a certain temperature, and then the low molecular products are determined	solid state
X-ray diffraction pattern analysis	Using the X-ray diffraction formed by crystal, the structural analysis method of the spatial distribution of internal atoms in matter is carried out.	solid state

2.3.4(a) Elemental analysis

As an instrument that can analyze the elements contained in substances, except for metal samples such as steel, aluminum and other alloys, elemental analyzers are widely used to identify the elements existing in organic substances and determine their contents. For example, the content and characteristics of organic carbon in soil not

only affect the physical and chemical properties of soil, but also closely related to the biological properties of soil. Therefore, it is necessary to analyze and study the composition of elements such as C, H, O, N and S in soil organic matter, especially the relative contents of C, H and O. The results showed that the content of C increased, and the ratio of H to H/C decreased in the middle stage of composting. Due to the action of microorganisms, the fatty substances were degraded and fragrant. As a basic analytical method, elemental analysis can be further combined with other analytical methods to analyze the changes in the chemical structure of samples.

2.3.4(b) Fourier transform infrared spectroscopy (FTIR)

Fourier infrared spectroscopy (FTIR) is one of the main methods for qualitative analysis of chemical structure of organic compounds (Tang, 2016). According to the peak shape, peak intensity and peak position of the infrared absorption curve, we can judge the possible chemical functional groups in organic matter. Fourier infrared spectroscopy can be used not only for the analysis of pure substances, but also for the analysis of mixed samples in materials, food, medicine, energy, biology, medicine and other fields. The part of the infrared spectrum below 1500 cm^{-1} is usually called the infrared fingerprint area, also called the infrared sensitive area (structure). The position of the infrared characteristic absorption peak of the same functional group changes little in different compounds.

Carboxyl, hydroxyl and carbonyl were the main functional groups of humus, and the molecular weight, aromatization degree and main functional groups of humus varied with different extracts. Using infrared spectroscopy to study the adsorption of atrazine on minerals, humus and soil, the results showed that the adsorption was mainly caused by hydrogen bond between nitrogen atoms and adsorption sites

(Schmeide et al., 2012; Sabah et al., 2017; Erhayem et al., 2013). Infrared and nuclear magnetic resonance techniques are used to study the effects of different fertilization treatments on the composition of humus groups. The results showed that the humic acid structure with chemical fertilizers is younger and simpler.

The Fourier transform infrared spectrum of humic acid samples is shown in Figure 2.5. It can be seen that different humic acid samples show very similar infrared spectral characteristics: strong and wide association -OH stretching vibration absorption at 3500-2500 cm^{-1}, 3000-2800 cm^{-1} fat C-H telescopic vibration absorption (vC-H); C=0 stretching vibration absorption of carboxyl and carbonyl functional groups at 1710 cm^{-1} (vC=O). At 1630 cm^{-1}, the absorption peaks of the skeleton vibration of aromatic ring, such as C=C absorption (vC=C), H bond association, C=O absorption (vC=O) and amide bond, are overlapped with each other. The O-H bending vibration of alcohols or carboxylic acids and the C-O stretching vibration peak (vC-O) of phenols are included at 1400 cm^{-1}. C-O stretching vibration (vC-O) and O-H deformation vibration at 1250 cm^{-1}, which are mainly carboxylic acid functional groups; And the O-H stretching vibration at 1050 cm^{-1}. These similar FT-IR spectral characteristics indicate that humic acid molecules from different sources have similar structural composition and functional group information.

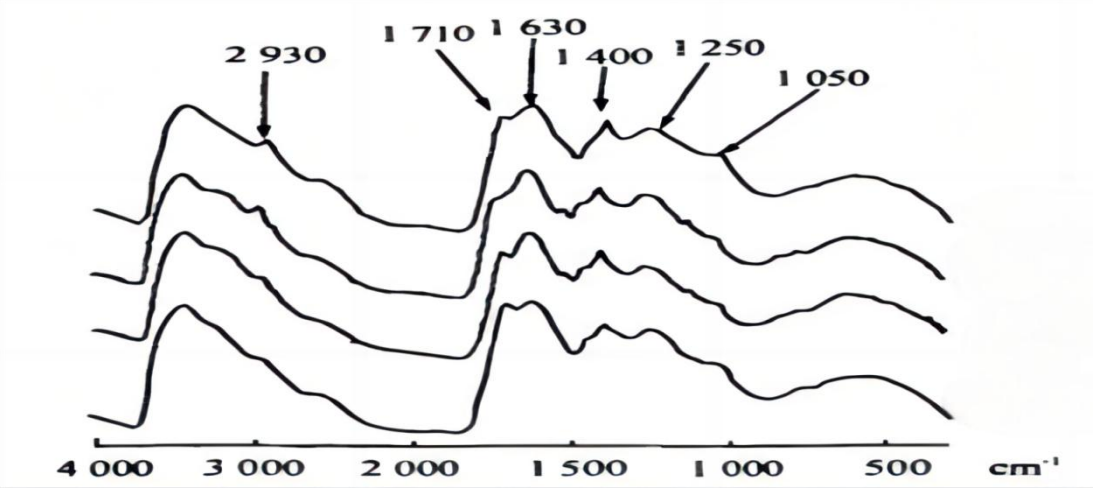

Figure 2.5 FT-IR spectra of 4 humic acids (Song et al.,2006)

2.3.4(c) Nuclear magnetic resonance (NMR)

Among many analysis methods, ^{13}C-NMR is one of the most effective methods to study the material structure developed in modern times, and it is also the most powerful methods to evaluate the organic matter of compost. Compared with other methods, nuclear magnetic resonance analysis, as a non-destructive spectral analysis technique, can be directly used for qualitative and quantitative determination of solid substances without destroying the sample structure. Carbon is the most important element in humus, so the study of ^{13}C NMR is of great significance to understanding the structure and composition of humus. Researchers can directly analyze the composition and structure of humus without extraction, and the aromatic C signal can be clearly distinguished from carboxyl, ether, methoxy and aliphatic C in the map, and the aromatization degree of C can be determined.

The solid CP/MAS^{13}C-NMR spectrum of humic acid samples is shown in Figure 2.6. It can be seen that all humic acid samples have detected seven NMR peaks, namely methyl/methylene, methoxy, carbohydrate, alkyl-substituted aromatic carbon, aromatic carbon connected with oxygen, carboxyl carbon and carbonyl carbon, etc. The integrated area and percentage of each peak are shown in Table 2.4. These peaks can be attributed to four different structural bands: Oxygen-containing fatty carbon; Aromatic carbon; Carboxyl carbon/Carbonyl carbon. This reflects that the four humic acids have similar chemical structures, mainly composed of aromatic carbon, fatty carbon and oxygen-containing groups. However, due to the different biological sources and diagenetic environments, each humic acid has its own structural characteristics, and the content of different structural units is obviously different.

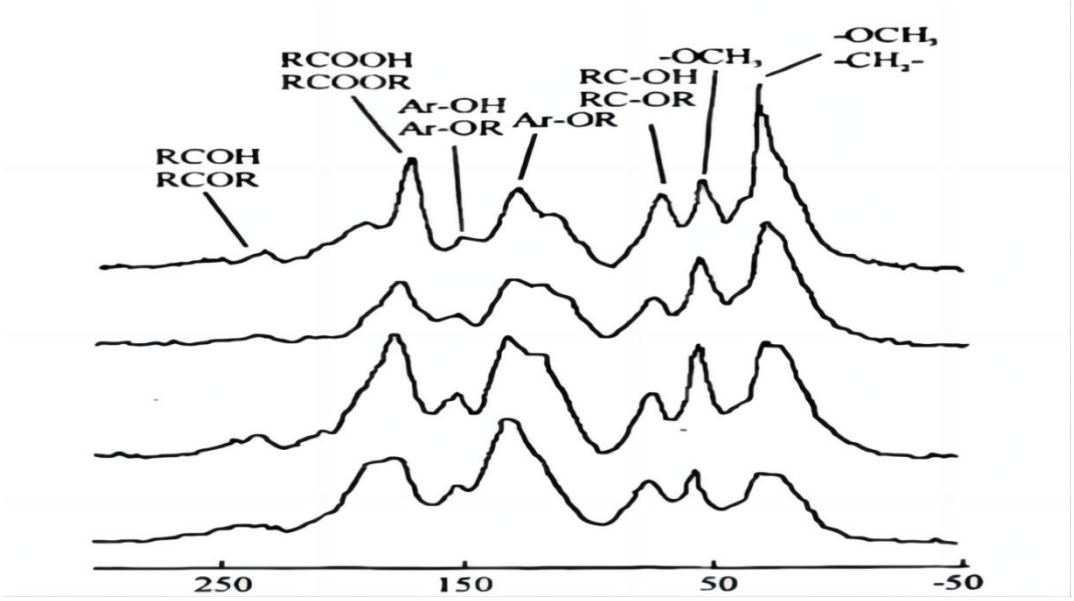

Figure 2.6 CP/MAS^{13}C-NMR spectra of four humic acids (Song et al., 2006)

Table 2.4 Estimation of various carbons type and typical peak ratios in the whole humic acid by ^{13}C—NMR spectr**a** (Song et al., 2006)

Sample	Percentage of functional group integral area %				Characteristic peak ratio		
	Fatty carbon	Oxygen-containing fatty carbon	Aromatic carbon	Carboxyl/ carbonyl carbon	Indicator 1	Indicator 2	Indicator 3
Humic acid-1	33.3	22.3	28.9	15.5	0.52	0.57	0.40
Humic acid-2	35.1	20.0	27.8	17.0	0.35	0.66	0.51
Humic acid-3	24.4	16.4	32.5	26.7	0.26	0.89	0.36
Humic acid-4	18.1	17.1	36.2	28.6	0.31	0.79	0.25

2.3.4(d) 3D excitation-emission matrix (3D-EEM)

Three-dimensional fluorescence spectroscopy is a non-destructive analysis technique, which can be used to characterize the degree of humification. The method has the advantages of simple method, high sensitivity, strong selectivity, small sample consumption and low detection limit. In addition, there is no need to add any chemical reagents before analysis, and only simple filtration is needed to determine the humification degree of compost samples. It has been reported that a large amount of information provided by three-dimensional fluorescence spectrogram can be used to the maximum extent by using three-dimensional fluorescence full spectrum analysis, and then quantitative research can be carried out better. At the same time, this technology can also overcome the problem that the chemical method will contain non-humic substances and have low detection sensitivity.

The three-dimensional fluorescence spectra of humic acid components with the same molecular weight and unclassified humic acid are shown in Figure 2.7, which shows that humic acid and its fractionated components all show fluorescence intensity when the excitation wavelength is 250-340 nm and the emission wavelength is 400-500 nm, and the excitation wavelength and emission wavelength corresponding to the strongest peak are 270 nm and 460 nm respectively, which is consistent with the characteristics of humic acid. The fluorescence intensity of humic acid fractions is negatively correlated with their molecular weight, which may be due to the higher aromaticity of high molecular weight fractions. This situation can be explained from two aspects: on the one hand, high molecular weight humic acid with higher aromaticity also contains more substituents, and self-quenching will occur both intramolecular and intermolecular. On the other hand, it can be clearly seen from Figure 2.7 that with the decrease of molecular weight, the fluorescence peak shifts