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Humic substances and its electron transfer capacity during composting: A review

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Abstract: Humic substances (HS) are important product during composting, which play a key role in the maturity of compost, and its electron transfer characteristics have significant applications in the reduction of pollutants. We focus on the structure and composition characteristics and formation mechanism of HS during the composting. The effects of composting raw materials, additives, microorganisms and oxygen supply conditions on the formation of HS were reviewed. The electron transfer mechanism of compost HS was summarized as well as the structural and environmental factors affect the electron transfer of HS. The applications of HS in soil pollution control and water treatment are introduced. The research directions of HS formation mechanism, electron transfer mechanism and its application are also prospected.

1 Introduction

Due to easy operation, composting is widely used for the treatment of organic solid waste, compost can be used for soil conditioning and nutrient supply [1]. HS are the main component of compost. It is formed by a series of complex biochemical processes that organic matter transforms during composting [2]. Including humic acid (HA), fulvic acid (FA) and humin (HU). Quinone is the main functional group compare with others which with redox characteristics in HS [3]. HS are able to accept and supply electrons, so it be used as an electron shuttle to transfer electrons. HS act as an electron shuttle could enhance the rate of electrons transmit during microorganisms reduced pollutants compared with direct pollutants reduction [4]. The formation process of HS and its electron transfer capacity vary with environmental conditions such as temperature, pH, microbial activity, and illumination [5]. In recent years, the electron transfer characteristics of HS have important applications in bioremediation of heavy metal and organic pollution, water treatment, and soil improvement [6].

In this study, the composition and characteristics of HS and its formation mechanism are reviewed. We sum up the electron transfer capacity and applications of HS. The mechanism of HS as an electron shuttle mediates electron transfer between microorganisms and oxidized minerals is summarized. We also conclude the influencing factors of the HS formation and the electron transfer process. Pointing out the deficiencies of the present research and further research and applications about HS.

2 Composition and structure and formation mechanism of compost HS and its influencing factors

2.1 Composition and structural of HS

HS are acidic macromolecule organic substance with dark brown. HS have mixed nature of aliphatic and aromatic, include C, H, N, S and O elements. HS contain carboxyl, carbonyl, alcohol hydroxyl, phenolic hydroxyl, methoxy, quinone, sulfonic acid and amino functional groups (Table 1), among which quinone and phenolic hydroxyl have redox effects. According to the different solubility of HS in acid and alkali solution in operation, it can be divided into HA, FA and HU [7].

The content and structural characteristics of HS are constantly changing during composting. HA increased slightly in composting, while FA decreased significantly [8]. The content of carboxyl and phenolic hydroxyl in FA and HA are less than HU, however HA has greater heterogeneity of carboxyl and phenolic hydroxyl than FA [9]. HU is insoluble in acid and alkali and is extracted difficultly. At present, there are few studies on the composition and structural characteristics of HU during composting, which may be used as the further research aspect.

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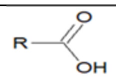
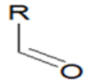
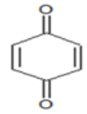
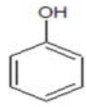
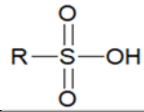
Table 1 The functional group names and structural formulas of HS.

Name	Structural formula	Name	Structural formula
Carboxyl		Methoxy	R-OCH_3
Carbonyl		Quinone	
Alcoholic hydroxyl	-OH	Amino	-NH_2
Phenolic hydroxyl		Sulfonate	

2.2 Formation mechanism of HS

Composting is accompanied by the decomposition of organic matters and the formation of HS. The organic matters change from unstable state to stable HS during composting [2]. In regard to the formation mechanism of HS in the composting process, there are two main theories. (1) Lignin theory: with the action of microorganisms, lignin side chains are oxidized to lignin derivatives (such as 3,4-dimethoxybenzoic acid methyl ester, 3,4,5-trimethoxyacetophenone, etc.), such derivative monomers through polycondensation or combine with other nitrides to cause the formation of HS [2]. (2) Polyphenol theory: organic matters generate aldehydes and phenols under the action of microorganisms, and are further converted into polyphenol compounds. Polyphenol compounds and small organic molecules are condensed into HS [7].

Lignin can provide more stable phenolic compounds as precursors for forming HS and building the core of HS [10]. Wu et al. [11] showed that polyphenols are important precursors to form HS. At present, the specific material conversion method and formation mechanism about the formation of HS are not clear, so further research is still needed. The formation pathway of HS during composting is shown in Fig.1.

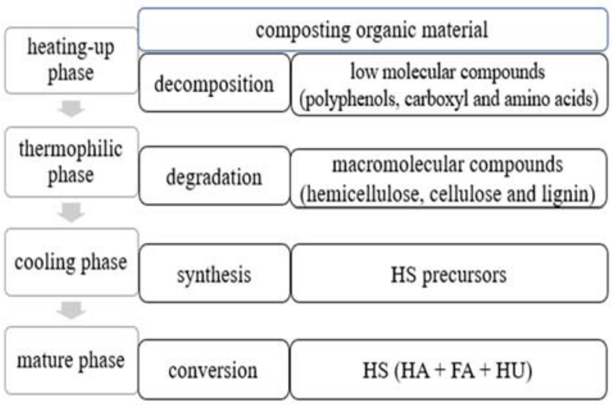


Fig. 1. Formation pathway of HS during composting [12].

2.3 Influencing factors of HS formation

The formation of HS is affected by compost raw materials, additives, microorganisms and oxygen supply conditions. (Table 2). (1) Different composting raw materials lead to differences in the degree of humification and structure of HS. The maturation time of Chicken manure compost is lower than sludge, and its rate of organic matter loss is higher than tomato rhizome [13]. (2) Most compost raw materials cannot be composted separately. Adding additives helps to improve microbial activity and accelerate the humification process. Fan et al. [14]found that when mushroom waste was added during the composting of kitchen waste, the C/N decreased from 22 to 16, and the GI (seed germination index) increased from 53% to 111%, which improved the compost maturity. (3) The change of microbial abundance and community will affect the decomposition and humification of organic matter [15]. Jurado et al. [16]believed that inoculation of polymer-decomposing microorganisms in lignocellulose compost boosted the degradation rate of hemicellulose, cellulose and lignin by 28%, 21% and 25%, respectively, and that humification was more intense. (4) Oxygen supply affect microbial activity. Low ventilation is not conducive to the decomposition of organic matter. When the ventilation is 0.68 L min^{-1} kg^{-1}, maturity and the degree of HS condensation and aromatization are increased [17].

In addition, the temperature, moisture content and pH will impact liveness of microorganisms and the degradation of organic matters, thus affecting the formation process of HS [12]. Various factors have a comprehensive effect on the formation of HS, and the formation of HS can be promoted by adjusting its parameter values. In the actual application process, composting equipment and methods may also affect the formation of HS, and need further the study.

Table 2. Influencing factors of HS formation during composting.

Influencing factor	Species	Effect on humification	References
Raw material	Chicken manure, food debris, sludge, tomato rhizomes	Chicken manure compost has the highest maturity and sludge compost has the longest maturation period.	Wang et al. [13]
Additive	Fish pond bottom mud	Improve C/H and O/C, enhance the degree of humification.	Zhang et al. [18]
Microorganism	Phanerochaete chrysosporium	HS content increased by 55.4%, the HS aromatic structure increased.	Chen et al. [19]
Oxygen supply	Ventilation capacity 0.45, 0.68, 0.90 L/min	Ventilation rate is 0.68 L/min with the highest degree of humification.	Ge et al. [17]
Temperature	46,30℃ temperature pretreatment	Organic matter degradation and compost maturity are higher by 46℃	Kianirad et al. [20]
Moisture content	Initial moisture content 50%, 60%, 70%	50% and 60% moisture content organic matter degrade faster	Tiquia et al. [21]
pH	pH 6-7,7-8,8-9	pH 6-7, microbial activity highest	Nakasaki et al. [22]

3 Electron transfer mechanism and its influencing factors of compost HS

3.1 Electron transfer mechanism

HS can be used as electron acceptor of microorganisms and electron donor of oxidized minerals, it is helpful to enhance rate of electron transfer between microorganisms and oxidized minerals [6].

The electron transfer of HS between microorganisms and oxidizing minerals mainly consists of three forms. (1) HS contact with C-type cytochrome (cyt) by diffusion, thereby transferring electrons between microorganisms and oxidizing minerals (Fig.2a) [23]. (2) Small molecular electron shuttle can obtaining electrons from the periplasm directly and bringing them to extracellular electron acceptors by across the cell membrane and entering cell (Fig.2b) [24]. (3) MR-1 bacteria can secrete flavins which combine with membrane proteins to form a complex of cyt and semiquinone when changing in potential. The complex of cyt and semiquinone alters the surface potential of microorganisms and affects the electron transfer process with the changes of biological metabolism (Fig.2c) [25]. However, the mechanism of cyt and semiquinone complex transfers electrons and the effect about electron transfer process which still need further study.

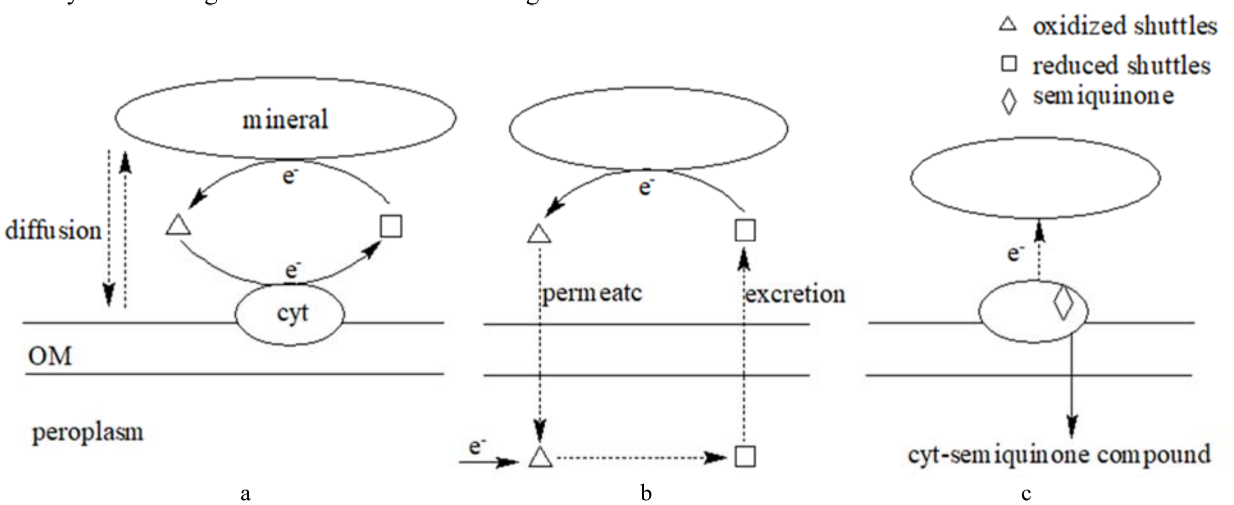


Fig. 2. Three electron transfer methods. a. shuttles contacts cyt; b. shuttles penetrate into the cell membrane; c. shuttles combined with cyt. (OM: outer membrane; cyt: cytochrome of the electron transfer system)

Taking process of Fe (III) mineral reduction as an example. The electron transfer process of HS between microorganisms and Fe (III) minerals includes two steps: in the first step, microorganisms transfer electrons to HS which are reduced. In the second step, the reduced HS transfer electrons to the end electron acceptor of the Fe (III) mineral which is also reduced [26]. After the two steps, the HS are not consumed, while are once again reduced by microorganisms, as electron donor of Fe (III) mineral, and then electron transfer was conducted again (Fig.3)[3].

In the first step, the electrons are transferred from the inside of microbial cell to the outside by the quinone group and the cyt, with the cytoplasmic oxidoreductase through the cytoplasmic membrane and periplasm to reach the outside of the cell membrane, the HS obtained electrons are reduced. Gescher et al. [24] found that soluble electron acceptors enter the outer membrane of the cell to obtain some electrons from the periplasm, while insoluble electron acceptors carry electrons through the cyt to the outside of the cell membrane to obtain electrons. This process is not achieved by a single cytochrome. Kulikova [27] believe that HS and AQDS (HS analogue) have the property of entering the cell to obtain electrons, but the mechanism needs to be more fully experimentally verified. At present, most research show that the electron shuttles obtain electrons outside the cell membrane mainly.

In the second step, the reduced HS transfer electrons to the terminal electron acceptor of the Fe (III) mineral through active diffusion and then once again acts as the electron acceptor of the microorganism [28]. Whether there are other transfer mechanisms requires further study.

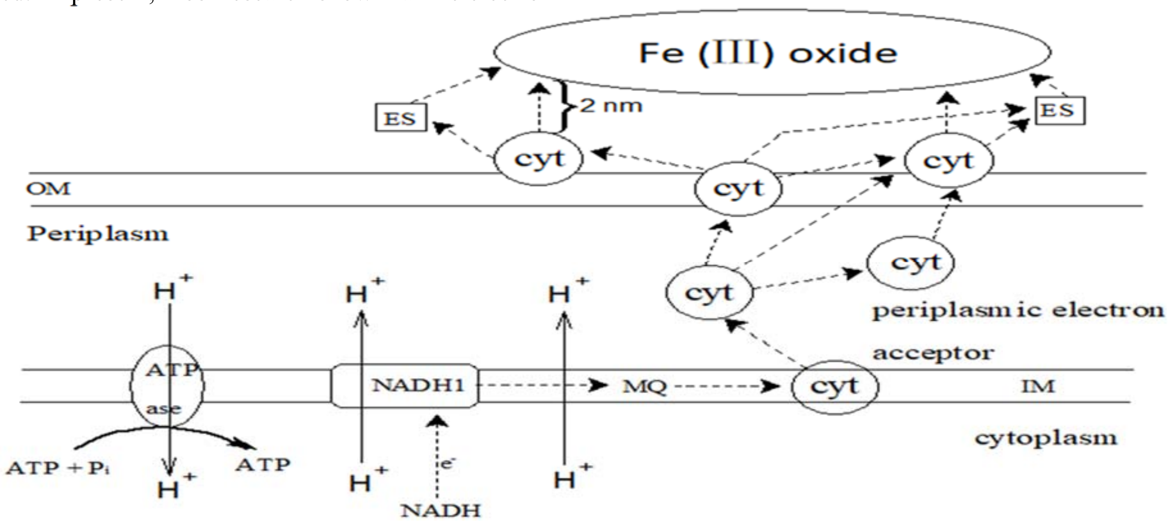


Fig.3. Electron transfer process of microbial reduction of Fe (III) minerals by electron shuttle [3].
(ES: electron shuttle; OM: outer membrane; IM: inner membrane; MQ: menaquinone; NADH / NADH1: dehydrogenase; cyt: cytochrome of the electron transfer system)

3.2 Influencing factors of electron transfer in compost HS

3.2.1 Structural factors

The higher hydroxy content in compost HS is beneficial to supply electrons, and it is good for obtaining electrons with more carboxyl ingredient [29]. Electron spin resonance measurements are performed when HS receive electrons, the results indicate that the quinone group is the main functional group that receives electrons during the reduction of microorganisms [30].

In addition, non-quinone functional groups also contribute to electron transfer capacity of HS. The H_2/Pd electrochemistry system was used to measure the electron transfer capacity of HA, it was found that the non-quinone functional groups in HA can account for 25%-44% of the total electron transfer [30]. The functional groups include nitrogen and sulfur element in HS also have obvious capacity to transfer electrons, for instance 3-methylthio-propionic acid, n-methylaniline and dimethyl sulfone [31]. Various functional groups in HS have capacity to transfer electrons. Confirming the degree of influence of different functional groups on HS electron transfer capabilities that can help to understand their electron transfer process.

3.2.2 Environmental factors

HS electron transfer capacity is affected by various factors, such as temperature, pH, oxygen supply, illumination and microbial activity and so on. Different environmental factors have diverse effects on electron transfer capacity. (1) The HS electron accept capacity increases, while supply capacity decreases as the temperature goes up. HS electron accept capacity enhance by 54%- 74%, while its electron supply capacity go down by 40%-56% when the ambient temperature change from -2 °C to 12 °C [32]. (2) The electron transfer capacity of HS depends on the pH, and single electron transfer process between the quinone, semiquinone and hydroquinone in HS is affected by the pH [33, 34]. The electron transfer capacity of HS increases with the growth of pH [35]. (3) Oxygen has protective effect on some redox sites of HS which result in the electron transfer capacity of HS is weakened, only few functional groups could transfer electrons [36]. (4) Illumination can cause photooxidation lead to HS lose phenolic hydroxyl in the early stage of photooxidation, thus electron-donating capacity will decline, however, it has little effect on its accept electrons capacity [37]. (5) Microorganisms are the main electron supplier of HS, and energy during microorganisms reduced HS can support the growth of microorganisms. Ten key species of HS-reducing determine the component of redox active group in HS, which affects its electron transfer capacity [38].

In addition, the concentration of oxidizing minerals and other electron acceptors present in the environment can also affect the electron transfer capacity of HS. The HS electron transfer capacity is affected by a variety of factors. Through scientific regulate and control key influencing factors, the HS electron transfer capacity will be maximized.

4 Applications of electron transfer of compost HS

4.1 Applications in pollutants reduction

There are many insoluble oxidized heavy metals in contaminated soil. HS could act as electron shuttle to promote the reduction of Fe (III), Cr (VI), Cu (II), Ag (I) and Cd(VI) and accelerate their conversion from insoluble oxidized state to relatively easy reduced state [39]. HS can also reduce Hg (II) to elemental mercury [40]. HS can also promote the reduction of Se (IV) and Te (IV). Reduction of heavy metals by HS that provides a new method for remediation of soil contaminated by heavy metals.

HS as electron shuttle could reduce organic pollutants such as hexachloroethane and nitrobenzene. HS reduce hexachloroethane to the only product tetrachloroethylene, and the reduction of HS by electrochemical methods can improve its capacity on reducing hexachloroethane [41]. Nitrobenzene can be reduced to anilines by HS. In anaerobic environment with a reduction potential less than -200mV, reduction of nitrobenzene by HS is an important way for its non-biological degradation [42].

4.2 Applications in water treatment

The use of coagulant increases the aluminum content in tap water. Orthophosphate can cause aluminum ions to form precipitate and be removed. The addition of HS will promote the precipitation of aluminum by orthophosphate [43]. The decoloration of azo dye wastewater requires a long hydraulic retention time in engineering. Adding HS can accelerate the reduction of azo dye and shorten the hydraulic retention time [6]. Whether HS can promote the removal of other industrial wastewater can be used as a research direction.

4.3 Other applications

HS as a hydrogel material have a higher adsorption capacity for heavy metals for the removal of Pb(II), Cu(II) and Cd(II) and are of great significance for the removal of toxic heavy metals [44]. HS act as an electron acceptor during the anaerobic digestion and acid production stage, accepting electrons from acetic acid to prevent its conversion into methane, reducing the efficiency of methane production by 52% and reducing greenhouse gas production [45]. The addition of HS enhance the power density and coulombic efficiency of microbial fuel cells by 60% and 78%, respectively [46].

5 Future perspectives

(1) The formation mechanism of composting HS is complex and affected by many factors. The path of HS formation by organic matter is unclear during composting. It is important to understand the formation path of HS and the role of influencing factors in regulating HS formation. (2) The electron transfer capacity of HS is affected by many factors. It is significant to confirm the degree of influence of different factors on the electron transfer capacity of HS, and it is necessary to deepen the research on this aspect. (3) The electron transfer capacity of HS has been applied to the remediation of contaminated soil and agriculture. In the future, the application of HS electron transfer capacity in other fields should be further studied.

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