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Characterization of Humic Acids and Fulvic Acids Derived from Sewage Sludge

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A comparative study of sludge humic acids and fulvic acids was carried out by means of elemental analysis, functional analysis, infrared spectroscopy and ultrafiltration fractionation. The result showed that humic acid was the main composition of sludge humic substances and fulvic acid was only one eighth of humic acid. 49.3 % of sludge humic acid and 88.9 % of sludge fulvic acid were extracellular substances. Sludge fulvic acid has more oxygen functional groups, fewer hydrocarbons, less aliphatic and aromatic structure and smaller molecular size than sludge humic acid. 37.7 % of fulvic acid had molecular weight of 30-50 kDa and 87.8 % of humic acid had molecular weight higher than 100 kDa. The fraction with small molecular sizes contained less condensed aromatic rings than aliphatic structures.

Key Words: Fulvic acids, Humic acids, Sewage sludge, Ultrafiltration.

INTRODUCTION

Sewage sludge is the main by-product of wastewater treatment plants (WWTPs). It is comprised of organic substances, inorganic mineral and water. The organic substances include protein, polysaccharide, humic substances, fat and nucleic acid^{1,2}. Humic substances can be divided into humic acids (HA), fulvic acids (FA) and humin (HU) according to their behaviours of dissolution in acid or alkaline solution. Humic acids play an important role during sludge treatment, because they are directly involved in the release of nutrients, cation exchange capacity, pH buffer capacity and heavy metals retention^{3,4}. The roles of humic acid are especially emphasized for sludge land utilization⁵. They can improve the metabolism of phosphorus and nitrogen in soil, help the assimilation of nutrients by plants and microorganisms and improve the aggregation and cationic exchange capacity of soil particles⁶. Hence, sewage sludge could be a potential source of humic fertilizers for soil improvement.

The composting process is a usual treatment used to transfer degradable organic substances, *e.g.*, protein, polysaccharide and fat, into more oxidized, unsaturated and aromatic humic compounds⁷⁻⁹. Thus, the humic acid with high molecular weight (> 25 kDa) increased, while that with low molecular weight decreased (< 1 kDa)⁷. Besides sludge composting, humification process also takes place during storage and other treatments. Sludge was found to be enriched in oxygen functional groups and aromatic rings in the course of storage and

fermentation, but the humification process was not completed due to the low value of free radical concentration and the unstable C/N ratio¹⁰. In the landfill of sludge mixed with municipal refuse, humic acid increased at first and then decreased because both humification and mineralization processes took place in landfill sites^{11,12}.

The evolution of sludge humic compounds was extensively analyzed during composting and other treatments. Though no much attention was paid to the formation and characteristics in waste activated sludge. It was recognized that the removal of humic substances in influents from WWTPs was mainly dependent on the absorption by activated sludge and the discharge of excess sludge, rather than the degradation by microorganisms². Some humic substances were also precipitated with flocculants and then removed in primary clarifiers. However, humic substances were difficult to be removed totally. It was reported that 3-28 % of the dissolved organic matter in effluents from WWTPs was humic substances, which, after hydrophilic acids, appeared to be the second most prevalent fraction in such effluents¹³. Thus, humic substances represent an important recalcitrant dissolved organic matter since they react with chlorine to produce carcinogenic by-products derived from disinfection protocols¹⁴.

The characteristic analyses of humic substances in excess sludge and primary sludge are important because their land utilization and removal from effluent are both influenced by their characteristics. For example, the distribution of humic substances in sludge floc reflects their absorption effect on

sludge floc, which determines the removal of humic substances in effluent. The molecular weight distribution of humic substances reflect their humification degree, which is directly involved in their land utilization. However, the relative information is rare as yet. Moreover, in most cases the analyses on sludge humic substances are concerning humic acid. The studies on sludge fulvic acid and its comparison with sludge humic acid are limited, although fulvic acid also play an important role during sludge treatment or utilization^{15,16}. Therefore, systematic analyses of sludge humic acid and fulvic acid were carried out with spectroscopic and chemical methods. Especially the quantities of extracellular and intracellular humic substances were measured, respectively and a full comparison between sludge humic acid and fulvic acid was implemented.

EXPERIMENTAL

The sewage sludge used in this study was collected from a local full-scale municipal WWTP. In the plant, a biological aerated filter process was applied to clean the wastewater. The excess sludge was the detached biofilm, which was mixed with primary sludge and then mechanically dewatered. The mixed sludge was collected and stored at 4 °C. The sludge sample had a water content of 98.0 %, a pH value of 7.1, a suspended solids content (SS) of 19.6 g/L, a volatile suspended solids content (VSS) of 10.3 g/L.

General procedure: The sludge sample was firstly centrifuged at 4000 rpm for 5 min and the supernatant was discharged. The sedimented fraction was used for humic acid and fulvic acid extraction according to the method recommended by the international humic substances society¹⁷. The extracted humic acid and fulvic acid were weighed and then used for further analyses.

In order to analyze the distribution of humic acid and fulvic acid in sludge floc, the extracellular polymers (EPS) were separated from the above sedimented fraction using formaldehyde and NaOH¹⁸. This method was recognized to be an effective way to extract extracellular polymers and simultaneously reduce the release of intracellular substances compared with other seven methods including EDTA treatment, glutaraldehyde treatment, sonication, resin absorption, heating, centrifugation¹⁹. According to the chosen method, the sedimented fraction derived from 50 mL sludge sample was diluted with 50 mL deionized water, then mixed with 0.2 mL of 36 % formaldehyde by magnetic stirring at 300 rpm for 0.5 h and finally mixed with 50 mL of 0.2 mol/L NaOH by magnetic stirring at 300 rpm for 4 h. The mixture was centrifuged at 7300 rpm for 10 min and the supernatant containing extracellular humic substances was used for further extraction and measurement of humic acid and fulvic acid according to IHSS method¹⁷. The intracellular fraction could be calculated by subtraction of the extracellular fraction from the total content of humic acid and fulvic acid.

Detection methods: Water content, suspended solid and VSS were measured according to standard methods²⁰. Sludge pH was measured with a pH meter (EUTECH Cyberscan510, Singapore).

In order to obtain the elementary composition of humic acid and fulvic acid, the percentage content of carbon, hydrogen,

nitrogen and sulfur was determined by means of an elementary analyzer (varioELcube, Elementar, Germany). Percentage concentration of oxygen was calculated by subtraction of the percentage content of carbon, hydrogen, nitrogen and sulfur from 100.

Total acidity (Ac) of humic acid and fulvic acid was determined by the barium hydroxide adsorption method and carboxyl groups (-COOH) by the calcium acetate method. The amount of phenolic hydroxyl groups (-OH) was calculated by subtracting the carboxyl groups content from the total acidity content.

Fourier transform infrared spectra of humic acid and fulvic acid were obtained using a Shimadzu FTIR-8400S spectrophotometer over a 4000-400 cm⁻¹ range.

The molecular weight (MW) distributions of humic acid and fulvic acid were detected by ultrafiltration method in an ultrafiltration cup (SMC, YuLing Ltd., Shanghai) under 0.2 MPa. The sample of humic acid or fulvic acid was dissolved in 0.005 mol/L NaCl solution and the pH was adjusted to 10 with 6 mol/L NaOH. The concentration of humic acid or fulvic acid in the solution was controlled lower than 3.5 g/L. A batch of the solution samples were filtrated with polysulfone ultrafiltration membranes (Sepro, U.S.) with different molecular weight cut-offs (MWCO), respectively. The MWCO of the membranes included 10, 30, 50 and 100 kDa and the concentration factor (CF) was 5. A series of permeation solutions were obtained with molecular weight lower than 10, 30, 50 and 100 kDa, respectively. The solutions were used for measurement of dissolved organic carbon (DOC) and ultraviolet absorbance (UV) and then the measured value was divided by 0.8 considering the penetration rate was about 80 %. Based on the calculated data, the dissolved organic carbon and UV distributions of humic acid or fulvic acid at different molecular weight intervals were calculated by subtraction. Dissolved organic carbon was measured with TOC-Vcph, Shimadzu. UV absorbance was measured in 1 cm quartz cells over a range from 800 nm to 200 nm using a Shimadzu UV-2450 spectrophotometer.

RESULTS AND DISCUSSION

Content and distribution: According to IHSS method, the contents of fulvic acid and humic acid in sludge were measured to be 25.0 and 201.4 mg/g VSS, respectively (Fig. 1). Humic acid was the main composition of sludge humic substances and it was almost eight times fulvic acid. The amount of extracellular humic acid was 49.3 % of the total humic acid and the amount of extracellular fulvic acid was 88.9 % of the total fulvic acid. During wastewater treatment processes, the humic substances derived from wastewater is removed due to biosorption of activated sludge². On the other hand, the humic substances in activated sludge may be utilized by microorganisms as a supplementary source of nutrients¹¹. Thus, the distribution of humic acid and fulvic acid in sludge floc was impacted by the effects of absorption and biodegradation. It was deduced that fulvic acid was more easily utilized by microorganisms and consequently, its content was far lower than humic acid and most of the fulvic acid existed outside cells.

TABLE-1
ELEMENTARY CONTENTS OF SLUDGE FULVIC ACID AND HUMIC ACID

	Elemental content (%)			Atomic ratio		
	C	H	N	H/C	O/C	C/N
Sludge fulvic acid	46.79 ± 2.40	7.89 ± 0.78	5.55 ± 0.62	2.02	0.62	9.84
Sludge humic acid	57.22 ± 0.24	8.46 ± 0.30	7.38 ± 0.06	1.77	0.32	9.04
Leonardite humic acid [Ref. 27]	60.93	3.53	0.94	0.69	0.41	75.6

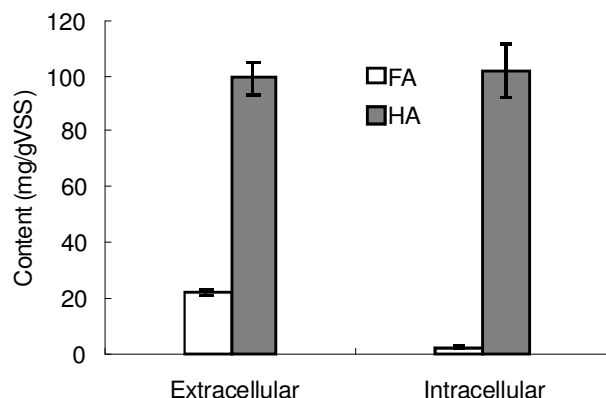


Fig. 1. Quantities of extracellular and intracellular humic acid (HA) and fulvic acid (FA)

Elemental composition, functional groups and FTIR spectra:

The elemental analyses (Table-1) showed that sludge fulvic acid had a lower carbon content and a higher H/C ratio than sludge humic acid. That meant sludge fulvic acid was less aromatic. Another obvious difference between fulvic acid and humic acid was their oxygen contents. The high O/C ratio of sludge fulvic acid indicated that it had more oxygen functional groups, such as hydroxyl and carboxyl²¹, than sludge humic acid. The commercial humic fertilizer commonly made from peat and leonardite and hence, it is valuable for sludge land utilization to compare the humic substances extracted from sludge with those extracted from more stabilized and traditional materials, *e.g.*, leonardite. It was notable that the H/C ratios of sludge humic acid and fulvic acid were both significantly higher than that of leonardite humic acid and the nitrogen contents of sludge humic acid and fulvic acid were also obviously higher than leonardite humic acid. The distinction can be attributed to their different humification degrees. On the whole, sludge humic substances were far from maturation compared with the more evolved organic materials.

The functional analyses (Table-2) verified that sludge fulvic acid had a higher content of oxygen functional groups, including carboxyl and phenolic hydroxyl, than sludge humic acid. The total acidities of sludge fulvic acid and humic acid and leonardite humic acid were all mainly derived from carboxylic acids. However, sludge humic acid and fulvic acid had lower contents of carboxylic and hydroxylic acid and lower ratios of OH/COOH compared with leonardite humic acid.

The lower total acidities reflected lower humification degrees of sludge fulvic acid and humic acid, which was consistent with the results of elementary analyses.

The information from FTIR transmissivity spectra (Fig. 2) also supported the results of the chemical analyses. The FTIR spectra of sludge humic acid and fulvic acid showed some similar bands and also some different bands. Based on the references^{8,22}, the bands in the spectra are the following:

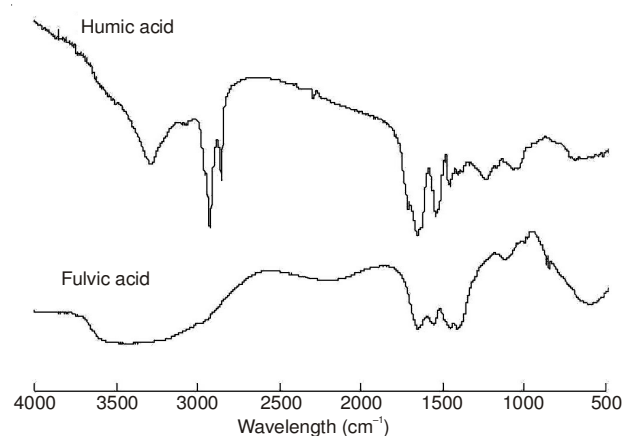


Fig. 2. FTIR transmissivity spectra of sludge fulvic acid and humic acid

A broad band at 3400-3300 cm⁻¹ occurs in the spectrum of sludge humic acid, which is assigned to H-bonded OH groups (alcohols, phenols and organic acids) and N-H groups. The band of sludge fulvic acid in the same place is broader, indicating more H-bonded OH groups.

The spectrum of sludge humic acid has an intense peak at 2930-2920 cm⁻¹ due to asymmetrical aliphatic carbon, especially CH₂ and a less intense peak at 2853 cm⁻¹ due to symmetrical aliphatic carbon CH₂. The spectrum of sludge humic acid has another broad band at 1080-1030 cm⁻¹, which is characteristic of aromatic ethers and possibly carbohydrates. In the spectrum of sludge fulvic acid, the peaks caused by aliphatic compounds (2930-2850 cm⁻¹) and carbohydrates (1080-1030 cm⁻¹) are not obvious, which indicates sludge fulvic acid is less aliphatic than sludge humic acid.

The spectrum of sludge humic acid has a broad band at 1670-1635 cm⁻¹ ascribed to olefinic and aromatic C=C, C=O (amide I) and a second less marked shoulder at 1550-1540 cm⁻¹ due to amide II and/or aromatic C=C. The spectrum of humic acid has a small band at 1470-1380 cm⁻¹ consisting of a

TABLE-2
AVERAGE FUNCTIONAL GROUPS CONTENT OF THE SLUDGE FULVIC ACID AND HUMIC ACID

	Ac (mmol/g)	-COOH (mmol/g)	-OH (mmol/g)	OH/COOH
Sludge fulvic acid	3.26 ± 0.08	2.84 ± 0.01	0.42 ± 0.07	0.15
Sludge humic acid	2.32 ± 0.04	2.02 ± 0.05	0.30 ± 0.01	0.15
Leonardite humic acid [Ref. 27]	7.12	4.38	2.74	0.62

weak peak at 1455 cm^{-1} corresponding to CH_3 and CH_2 and a second, more pronounced peak near 1410 cm^{-1} corresponding to several groups including phenolic OH, COO^- asymmetric stretch, stretching of *ortho*-disubstituted aromatic rings. Compared with humic acid, the lower peaks caused by aromatic compounds and amides ($1670\text{--}1635$, $1550\text{--}1540\text{ cm}^{-1}$) in the spectrum of sludge fulvic acid show that fulvic acid has a lower aromatic degree and N content, while the more intense peaks at 1410 cm^{-1} demonstrate that fulvic acid has higher contents of phenolic OH and COO^- .

The spectrum of sludge humic acid has a weak band at $1244\text{--}1224\text{ cm}^{-1}$ due to amide III or aromatic ethers, which is not found in the spectrum of sludge fulvic acid. Another weak band at $1130\text{--}1100\text{ cm}^{-1}$ due to alcoholic hydroxyl group is only found in the spectrum of sludge fulvic acid, which shows that sludge fulvic acid has more oxygen functional groups and less aromatic structure.

According to the above analyses, sludge fulvic acid had more oxygen functional groups and fewer hydrocarbons (lower aliphatic and aromatic degrees) compared with sludge humic acid. The results were accordant with the analyses of elemental composition and functional groups.

Fractionation by ultrafiltration: Sludge fulvic acid and humic acid are both composed of many different molecules. According to their molecular weights, sludge fulvic acid and humic acid can be divided into different fractions by a series of ultrafiltration membranes with different MWCO. The DOC ratio of every fraction is shown in Fig. 3. Most of fulvic acid organic carbon is derived from the fraction with molecular weight of 30–50 kDa, while most of humic acid organic carbon is from the fraction with molecular weight higher than 100 kDa. On the whole sludge humic acid has bigger molecular size than sludge fulvic acid. Similar results on humic acid fractionation were reported. For peat humic acid, the mass of the fraction with molecular weight higher than 100 kDa was 73.6 % of the total mass²³. For humic gleysol, the DOC of the fraction with molecular weight higher than 100 kDa was $59 \pm 7\%$ of the total DOC²⁴.

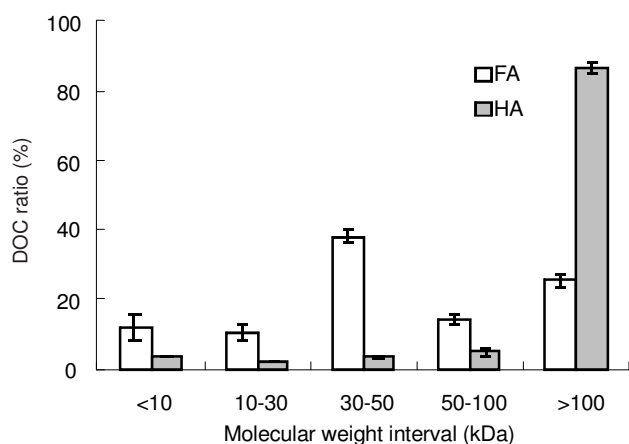


Fig. 3. DOC ratios of sludge humic acid (HA) and fulvic acid (FA) at different molecular weight intervals

The UV absorbance ratio $E_{2/3} = A_{250}/A_{365}$ is used to evaluate the aromaticity of humified materials²⁵. The degree of aroma-

ticity (DA) can be obtained by the following equation: $DA = 52.509 - 6.780E_{2/3}$ ²⁶. Table-3 lists the $E_{2/3}$ values and DA of each fraction. For either sludge humic acid or fulvic acid, the $E_{2/3}$ ratio decreases with the increasing molecular size on the whole, but the opposite trend occurs on the aromaticity degree. These results show that the fraction with small molecular sizes contains less condensed aromatic rings compared to aliphatic structures. Compared sludge humic acid and fulvic acid, it is verified that humic acid was more aromatic on the whole. This is consistent with the results of functional groups analysis.

TABLE-3
UV ABSORBANCE CHARACTERISTICS OF
SLUDGE HUMIC ACID AND FULVIC ACID

HS fractions (kDa)	Fulvic acid		Humic acid	
	$E_{2/3}$	DA	$E_{2/3}$	DA
<10	8.21	-	2.30	36.92
10-30	4.49	22.07	4.50	22.00
30-50	4.03	25.19	3.28	30.27
50-100	3.00	32.17	1.49	42.41
>100	2.70	34.20	2.74	33.93
Unfractionated	4.31	23.29	3.16	31.08

Conclusion

Humic acid was the main composition of sludge humic substances and fulvic acid is only 1/8th of humic acid. About half of the sludge humic acid is extracellular substances, while 90 % of sludge fulvic acid is extracellular substances. Sludge fulvic acid has more oxygen functional groups, less aliphatic and aromatic structure compared with sludge humic acid. The molecular size of sludge fulvic acid is significantly smaller than that of sludge humic acid. Most of fulvic acid organic carbon comes from the fraction with molecular weight of 30–50 kDa, while 90 % of humic acid organic carbon comes from the fraction with molecular weight higher than 100 kDa. The fraction with a smaller molecular size contained a smaller number of condensed aromatic rings compared to the number of aliphatic structures.

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