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Effect of ultrasonication on the humic acids extracted from lignocellulose substrate decomposed by anaerobic digestion

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Abstract

The effects of ultrasonication on the humic acids extracted from the lignocellulose decomposed by anaerobic digestion were investigated via ultraviolet (UV) absorbency, Fourier transform infrared (FT-IR), ^{1}H nuclear magnetic resonance (^{1}H NMR) spectra, gel permeation chromatography (GPC) and scanning electron microscope (SEM). The chemical modifications induced by ultrasonication mainly occurred in the carboxylic acid groups, the α-methylene groups on the aromatic rings, a variety of aliphatic carbons (mainly polymethylene), β-aliphatic carbon on the aromatic rings and α-carbon oxygen bond. The SEM micrographs showed that the fibrous structures of the humic acids tended to aggregate after ultrasonication. An increase in the average molecular weight (MW) of the humic acids after ultrasonication was also observed in the GPC analysis. These results indicate that the ultrasound could not decompose the humic acid substrates completely. However, the results suggest the potential use of ultrasound as a basis for pretreatment before use of other methods to dispose of humic acids in contaminated water.

Keywords: Environment; Down stream processing; Humic acids; Ultrasound

1. Introduction

Humic substances are among the most widely occurring natural products on the earth's surface. They are generally refractory to any substantial biodegradation, owing to their complicated chemical structures. The formation of indigestible humic substances during the anaerobic degradation of lignocellulosic wastes remains a problem as far as energy production and re-utilization of lignocellulosic wastes are concerned. Furthermore, pollution occurs instantly when water-soluble humic substances, such as humic acids, are discharged into aquatic environments. For example, humic acids can carry heavy metal ions, insoluble organic materials and xenobiotics and increase their solubility and motility in soil and water [1,2]. Moreover, they form trihalomethanes and exhibit other carcinogenic and mutagenic activity by reaction with chlorine dosed in water purification processes [3,4]. It is therefore imperative to treat these water-soluble humic substances resulting from the biodegradation of lignocellulosic wastes, before discharging them into aqueous environments.

In common water treatment processes, the removal of water-soluble humic substances has been accomplished by physical separation, such as coagulation followed by subsequent floc separation, ion exchange, adsorption on activity carbon, membrane filtration and chemical degradation methods such as using strong oxidizing agent [5], as well as ultraviolet (UV) irradiation [6,7] followed by biofiltration. These methods all have their advantages and disadvantages, but none of them has proved to be fully satisfactory in practice.

A further possibility is the use of ultrasound. Ultrasound between 20 and several hundred kilohertzs is known to create resonant cavities, which release energy during collapse in a process called cavitation [8,9]. Cavitational sites, commonly referred to as sonochemical hot spots, have been described in terms of two distinct regions, a gaseous core and a surrounding supercritical liquid shell ($T_c = 374\,^\circ$C; $P_c = 221$ bar) [10]. The reactive species including H_2O_2, HO_2, $H^\bullet$ and $OH^\bullet$, produced in the shell during cavitation, can give rise to radical chain reactions throughout sonolyzed media [8].

In contrast to other chemical (such as acid hydrolysis, oxidation), thermal or physical decomposition reactions (such as pyrolysis, X-ray or γ-ray irradiation), ultrasonic decomposition of organic waste is a stochastic process, where chain

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scissions near the center of the largest molecules are favored. It is commonly used to break up macromolecules in solution and has been applied to a wide variety of polymers including both water- and nonwater-soluble compounds [11–16].

While the authors previously demonstrated that ultrasonication can be applied to a broad range of organic pollutants in aqueous systems, including complex mixtures of chemicals in a waste streams, not all substrates are equally susceptible to sonochemical degradation [17,18]. For example, the characteristics of humic acids subjected to different environmental conditions could be significantly different [19]. This study was specifically conducted to investigate the effects of ultrasonication on the humic acids extracted from the lignocellulose substrates decomposed by anaerobic digestion.

2. Materials and methods

2.1. Extraction of humic acids

The humic acids were extracted from the humus from an anaerobic laboratory digester fed with lignocellulose residues (tobacco dust). The humus was mixed with 0.5 M NaOH under N_2 at a solid to liquid ratio of 3/20 (w/v). After leaving the treated slurry for 24 h in dark at room temperature, the supernatant was separated by centrifugation. The filtrate was precipitated at a pH of 1.17–1.50 with HCl, the precipitate was washed with 0.1 M HCl, followed by distilled water, then freeze–dried and stored in the dark at $-16\,^{\circ}$C.

2.2. Ultrasonication of humic acids

Freeze–dried humic acids were dissolved in 0.5 M NaOH solution to the concentration of 1.1×10^3 mg/l and the pH of the solution was adjusted to approximately 7.3 with 1 M HCl or 1 M NaOH (Merck). This solution was diluted by 50% with distilled water before every experiment. The ultrasonication set-up is shown in Fig. 1. Ultrasonication was carried out in a spherical glass vessel with a volume of 100 ml (45 mm i.d.). The ultrasound was generated by a 50 W sonicator (U50 control, IKA Labortechnik Staufen, UK) with maximum horn power output of 130 W/cm^2 (at ambient temperature and pressure) at 30 kHz via a titanium alloy rod (7 mm diameter), which was immersed 15 mm below the sonicated suspension. The effective ultrasound intensity transmitted to the suspension was approximately 230 db, as measured by a Reson TC4033 hydrophone. The pH and oxidation–reduction potential (ORP) of the solution were measured instantly after ultrasonication, using a PHM 82 standard pH/mV meter). Each 20 ml of treated solution and 20 ml of untreated solution was filtrated (0.45 μm cellulose acetate, Schleicher and Schuell) and analyzed for UV absorption within a few hours. The rest of the solution was immediately freeze–dried and stored in the dark at $-16\,^{\circ}$C for other analyses.

2.3. Physicochemical measurements of humic acids

Various aspects of the treated humic acids were measured as follows:

- The changes of UV absorbency at 230 nm (UV_{230}) [20] and 254 nm (UV_{254}) [21] resulting from ultrasonication were measured with Cary 1E UV-Vis spectrophotometer (series 94041466) using 1.0 cm quartz cells.
- The reactivity of humic acids with Fast Blue B (FBB) salt was measured with the Single Cell Kinetics Method on the Cary 1E UV-Vis spectrophotometer, following procedures, specified in the literature [22]. One milliliter of humic acid solution was mixed with 0.1 ml of 4.21 mM FBB solution. The increase in the absorbency at 530 nm (UV_{530}) was recorded twice from 0 to 30 s. The reactivity was indicated by the rate of the increase in UV_{530} over the initial 12 s. The E_4/E_6 ratio of samples was determined by calculating the ratio of the UV absorbency

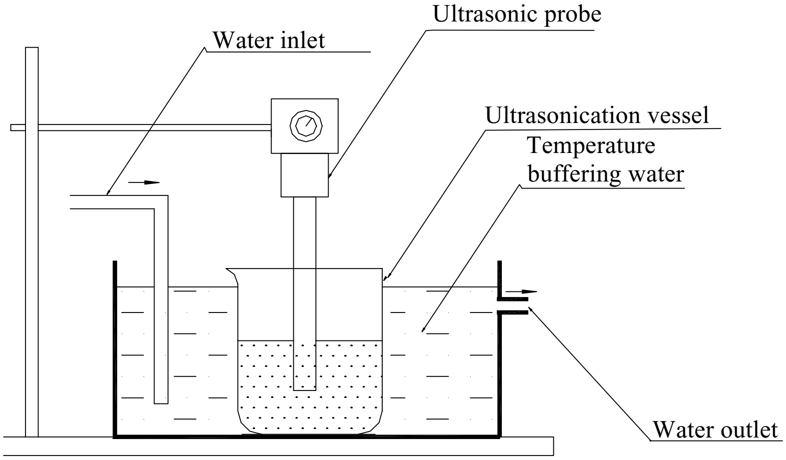

Fig. 1. Experimental set-up.

at 465 and 665 nm (UV_{465}/UV_{665}) to determine changes in the molecular structures of the humic acids in the solution according to the method of Chen et al. [23], using the Cary 1E UV-Vis spectrophotometer.

- Fourier transform infrared (FT-IR) spectra of humic acids in KBr pellets (1 mg freeze–dried humic acids in 300 mg KBr) were recorded on a Mattson GALAXY 2020 FT-IR spectrometer.
- ^{1}H NMR spectra of humic acids in deuterium oxide (dissolving ∼20 mg of the freeze–dried humic acids in 0.5 ml deuterium oxide) were determined on a Varian VXR 300 NMR spectrometer.
- The effects of ultrasonication on the molecular size (weight) distribution of humic acids dissolved in tetrahydrofuran (THF) solutions were examined chromatographically by gel permeation chromatography (GPC) (linear UV detector at 240 nm, Phenomenex—Phenogel 100 and 500 Å, 300 mm × 7.8 mm columns, 20 μl injection volume).
- The effects of ultrasonication on the microstructure of the humic acids were examined using a Topcon ABT-60 scanning electron microscope (SEM). The freeze–dried humic acid samples were attached to aluminium stubs and coated with a layer of gold a few atoms thick.

3. Results and discussion

3.1. Effect of ultrasonication on the pH and ORP of humic acids in solution

The changes in the pH and ORP of the solution of humic acids treated by ultrasonication are presented in Fig. 2. The results show that the pH of the solution increased slightly from 7.30 to 7.42 and then dropped to 7.18 with an increase in the ultrasonication period. Little change in the ORP of the solution (less than 10 mV) was observed as a result of ultrasonication. The changes in the pH can possibly be attributed to the fact that when the aqueous solution is irradiated by ultrasound, water vapour present in the ultrasound bubble is split to yield $H^\bullet$ and $OH^\bullet$ radicals. Humic acid molecules present in the vapour phase or in the surrounding liquid of the collapsing bubbles are subject to direct attack by $OH^\bullet$ radicals, thus probably accelerating hydrolysis by several orders of magnitude in the presence of ultrasound. The acidic nature of humic acids is usually attributed to the ionization behaviour of –COOH and phenolic –OH groups. The change in pH of the humic acid solution by ultrasonication implies that the ionization behaviour of the acidic functional groups of humic acids in ultrasound field could be modified. Since, humic acids behave like weak-acid polyelectrolytes with a relatively high pH buffering capacity, changes in the pH of the humic acids would probably not be evident, even when the acidic functional groups of the humic acid molecules change. Oxidation, pyrolysis and/or the mechanical degradation of humic acid molecules could be expected in an ultrasonic field. Changes in the ORP of the aqueous humic acid solution can mostly be attributed to electron transfer in the system, and therefore the negligible change in the ORP of the humic acid solution suggests that no substantial electron transfer was stimulated between the radical species in the ultrasonic field and the reactive functional groups of the humic acids.

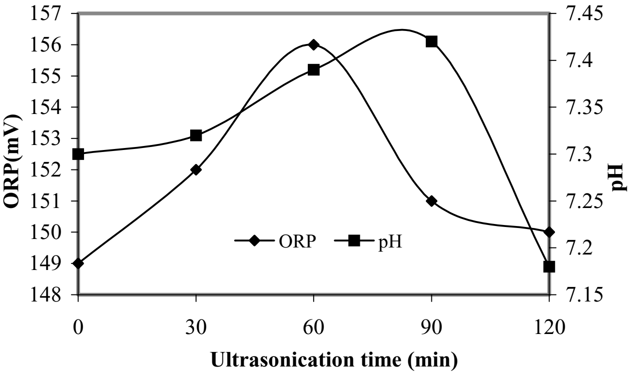

Fig. 2. Effects of ultrasonication on the ORP and pH of the humic acid solution.

3.2. The effects of ultrasonication on the UV absorbency of humic acids

The changes in the UV_{230} and UV_{254} absorption levels are given in Fig. 3. The results show that the UV_{230} and UV_{254} absorption of the humic acid solution first increased with ultrasonication, then decreased after 60 min of ultrasonication. The absorption of radiation in the UV-Vis region of the electromagnetic spectrum arises from electron transition from bound states (outer valence orbitals) to excited electron states. In humic acid molecules, these exceptionally low energy transitions are associated with the presence of chromophores, i.e. functional groups containing conjugated double bonds and sulphur, nitrogen or oxygen atoms with delocalized electronic orbitals. Groups which are not

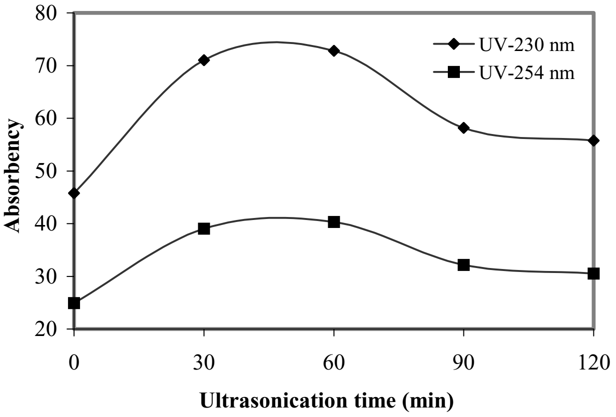

Fig. 3. Effect of ultrasonication on the UV absorbency of the humic acid solution.

chromophores, but affect absorption of chromophores, are called auxochromes, which typically include hydroxyl and amine groups. Despite the fact that the UV-Vis spectra of humic acids are generally featureless (the spectra are not shown here) owing to the extended overlap of absorption of a wide variety of chromophores affected by various substitutions, UV-Vis light absorption of humic acids appears to increase with an increase in: (a) the degree of condensation of the aromatic ring [24], (b) the total C content, (c) the molecular weight (MW), (d) the ratio of C in aromatic rings to C in aliphatic side chains [25], and (e) the pH of the solution [26]. The changes in UV_{230} and UV_{254} absorption as a result of ultrasonication suggest that the structure and/or contents of chromophores, such as OH-, COOH-substituted benzene rings, the intramolecular electron donor–acceptor complexes and complex unsaturated conjugated chromophores had changed in the ultrasound field. Comparing the changes in the UV_{230} and UV_{254} absorption (see Fig. 3) with the changes in the pH (see Fig. 2), it is clear that ultrasonication could induce changes in disassociation or protonation of carboxyl and phenolic hydroxyl groups of the humic acids. In addition, the macromolecular structure of the humic acids could have been altered, resulting in greater or lesser exposure of chromophores to light, while auxochromes also could have been introduced into aromatic rings.

3.3. The effect of ultrasonication on the reactivity of humic acids with FBB

FBB reacts with 1-naphthol and other hydroxylated aromatic compounds to form colored products which have absorption in the range of 530–618 nm [22]. The changes of reactivity of the humic acids with FBB are presented in Fig. 4. The results indicate that the reactivity of the humic acids with FBB initially increased with an increase in the period of irradiation, then decreased after 60 min of irradiation. This suggests that the ultrasonication probably initially stimulated the formation of 1-naphthol or other hydroxylated aromatic-like compounds in humic acids. However, these compounds gradually decomposed when ultrasonication was prolonged.

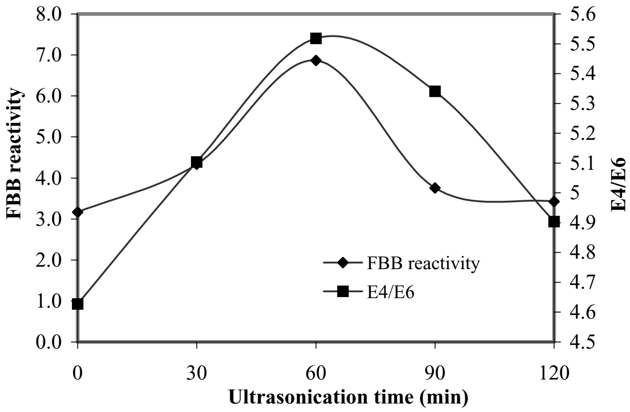

Fig. 4. Effect of ultrasonication on the FBB reactivity and E_4/E_6 ratio of the humic acids.

3.4. The effect of ultrasonication on the FT-IR spectra of humic acids

The changes in the FT-IR spectra of the humic acids are presented in Fig. 5. The associated dominant peak assignment [27,28] and the integrated areas are listed in Table 1. The FT-IR spectra of five humic acid samples showed the presence of oxygen-containing functional groups. They are mainly the: (a) broad bands around 3002–3820 cm^{-1}, which could be attributed to the valence vibrations of hydroxyl groups (3400 cm^{-1}) and aromatic C–H bonds (3295–3006 cm^{-1}), (b) shoulders at 1771–1500 cm^{-1}, which could be attributed to the C–O stretch of C=OOH and stretches C=O of ketonic carbon, aromatic C=C, C–O stretch of COO– and hydrogen-bonded C=O, (c) peak region in 1200–956 cm^{-1}, which could be attributed to C–O, C–N and C–C stretch, and (d) peak region in 900–775 cm^{-1}, representing the oxygen (O) substitution arene groups, were characteristic for all samples. The absorption bands in the 2900 cm^{-1} region were usually superimposed on the shoulder of the broad O–H stretching band.

Fig. 5 shows that no remarkable shifts in these main bands were found after ultrasonication. However, some small peaks (comparing spectra a, b and c), such as the peaks around 2900 cm^{-1} (the asymmetric and symmetric stretching vibrations of aliphatic C–H bonds in CH_3 and CH_2 groups), 2400 cm^{-1} (after 1 h ultrasonic irradiation), 1520 cm^{-1} (amide II or aromatic C=C stretch) and 1200 cm^{-1} (aliphatic C, O–H or C–O stretch of various groups) occurred in the spectra after ultrasonication. Table 1 shows that the intensity (integrated absorbency peak areas) of some absorption bands changed after ultrasonication. This suggests that a variety of chemical components, some chemical modifications in the molecular structure and changes in the functional groups of humic acids were probably induced by ultrasonication.

3.5. The effect of ultrasonication on the ^1H NMR spectra of humic acids

The ^1H NMR spectra of the initial humic acids and the 2 h irradiated humic acids are presented in Fig. 6(a) and (b). The chemical shifts and corresponding assignments [29,30] of absorbency peaks are summarized in Table 2. Fig. 6 shows that four sharp resonances at 0.88, 1.27, 6.71 and 8.43 mg/l were observed in the initial humic acid sample. Three sharp resonances at 0.85, 1.25 and 8.42 mg/l were observed in the humic acid sample treated by ultrasonication. It is obvious that the sharp signal at 6.71 mg/l disappeared after irradiation. This implies that some special aromatic group compounds (e.g. phenol) were decomposed by ultrasonication.

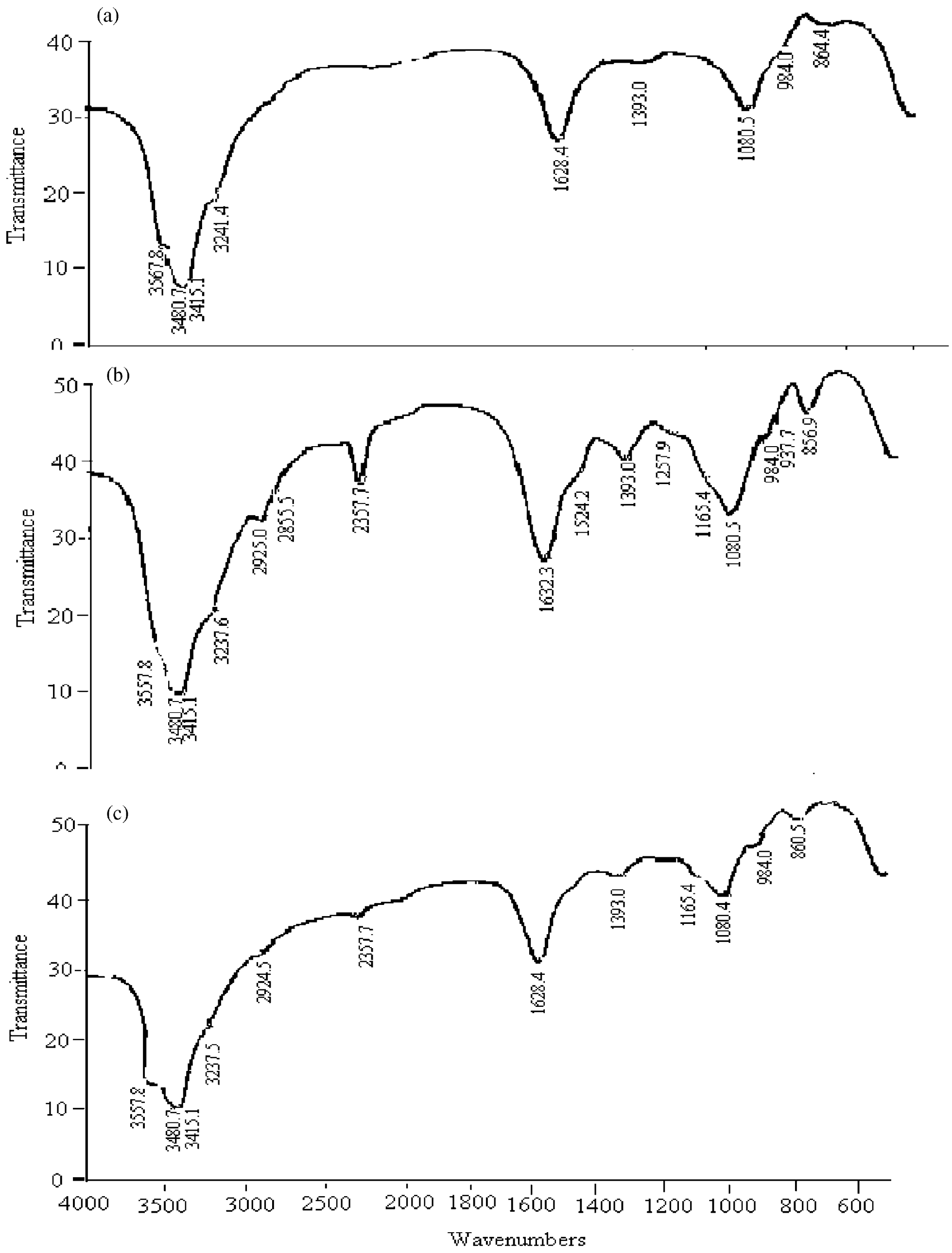

Fig. 5. FT-IR spectra of humic acids: (a) with 0 h of ultrasonication; (b) with 1 h of ultrasonication and (c) with 2 h of ultrasonication.

Table 1
Integrated peak areas of the FT-IR spectra of humic acids treated with different periods of ultrasonication

Function assignment	Wave number (cm^{-1})	Ultrasonication (min)				
		0	30	60	90	120
–OH–NH, aromatic CH stretch	3002–3820	177.969	107.834	177.548	128.867	141.351
–C=OOH, C=O of ketonic C, aromatic C=C, COO–, hydrogen-bonded C=O stretch	1771–1500	16.831	9.542	27.052	17.575	12.177
C–O or OH stretch	1200–956	7.177	3.437	6.277	3.659	3.423
O substitution arene	900–775	0.794	0.891	2.601	1.708	0.980

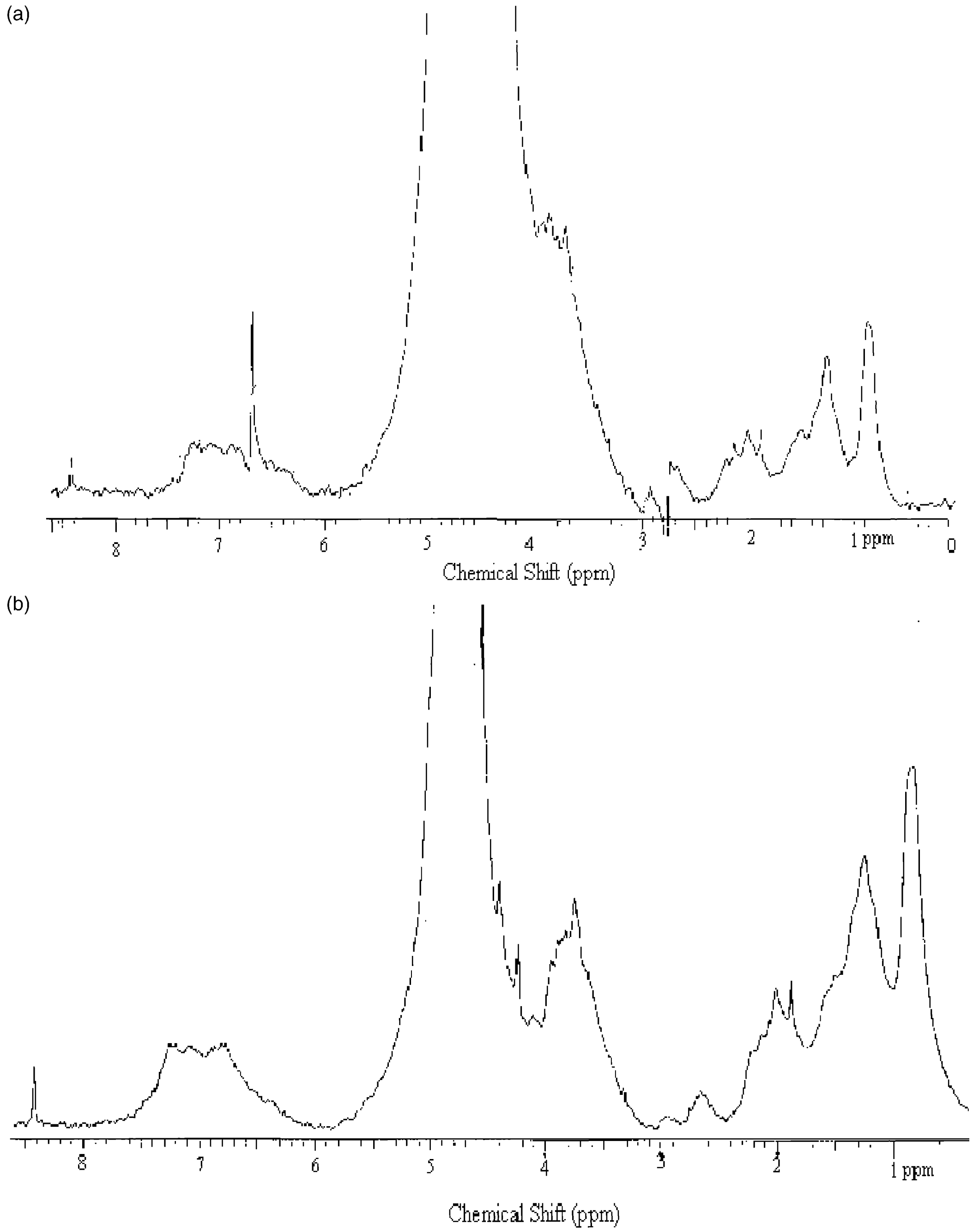

Fig. 6. Effects of ultrasonication on the ^{1}H NMR spectra of humic acids: (a) with 0 h ultrasonic irradiation and (b) with 2 h ultrasonic irradiation.

The signal at 2.431–3.135 mg/l (representing protons attached to α-carboxylic acid groups or protons attached to α-methyl and methylene groups on the aromatic rings) appeared after ultrasonication. In addition, Table 2 and Fig. 6 show that the spectrum at 1.4–1.8 mg/l, which arises from a variety of aliphatic carbons (mainly polymethylene protons and protons attached to β-aliphatic carbon on the aromatic rings) [31], and the spectrum at 3.0–4.4 mg/l, which arises from the protons attached to α-carbon oxygen bond, changed after ultrasonication. This indicates that the changes in the chemical structure of humic acids induced by ultrasonication took place mainly in the carboxylic acid groups or α-methylene groups on the aromatic rings and a variety of aliphatic carbons (mainly polymethylene), such as β-aliphatic carbon on the aromatic rings and α-carbon oxygen bond.

The spectrum at 8.3–8.5 mg/l (sterically-hindered protons of aromatics) showed no significant changes after ultrasonication. However, the resonance at 0.89 mg/l (representing the methyl groups of alkyl chains) shifted to 0.39 mg/l after ultrasonication. This confirmed that the terminal methyl groups of the methylene chains had been changed, especially

Table 2
Chemical shift ranges and tentative assignments of major ^{1}H resonance observed from humic acids ^{1}H NMR spectra

Chemical shift range (mg/l)		Integrated area		Tentative assignments
HA(0)	HA(1)	HA(0)	HA(1)	
0.559–1.062	0.393–1.022	24.661	40.298	Terminal methyl groups of methylene chains
1.062–1.439	1.022–1.743	24.761	51.709	Methylene of methylene chains; CH_2CH at least two carbons or further from aromatic rings or polar functional groups
1.439–1.758		11.806		Methylene of alicyclic compounds
1.758–2.429	1.744–2.430	17.851	23.117	Protons attached to α-methyl and methylene groups on the aromatic rings
	2.431–3.135		3.01	Protons attached to α-methyl and methylene groups on the aromatic rings; protons attached to the α-carboxylic acid groups
3.007–4.030	3.136–4.419	98.632	63.493	Protons attached to α-carbon to oxygen groups; carbohydrates
6.152–7.578	6.138–7.672	33.886	28.647	Aromatic protons including phenols
8.341–8.508	8.318–8.511	0.725	0.629	Sterically-hindered protons of aromatics

HA(0), humic acids without ultrasonication; HA(1), humic acids with 2 h ultrasonication.

in carboxylic acid groups, α-methylene groups on the aromatic rings and some aliphatic carbons (mainly polymethylene), whereas little change took place in the aromatic kernel structure of the humic acids.

For complex macromolecules such as humic acids, the observed broadness of the bands in the FT-IR spectra was probably a result of the extended overlapping of very similar absorption arising from individual functional groups of the same type, with different chemical environments. Although most groups of atoms vibrate with almost the same frequency irrespective of the molecule to which they are attached, this fact does not mean that the humic acids displaying similar FT-IR spectra, must have similar overall structures, but only that the net functional group and structural entities may be similar [32]. Therefore, the FT-IR spectra in Fig. 5 could only suggest that ultrasonication induced the changes in the chemical environments of the net functional groups and structural entities of the humic acid macromolecules. However, the ^{1}H NMR spectra in Fig. 6 confirmed that the chemical structures of the humic acids were changed by ultrasonication.

Table 3
The statistical distribution of molecular weights of the humic acids with different periods of ultrasonication measured with GPC

Ultrasonication (h)	M_W	M_N	M_Z	Polydispersity	10% at M_W	90% at M_W
0	465	408	593	1.1386	754	338
1.0	601	468	847	1.2860	1142	331
1.5	607	483	829	1.2561	1116	347
2.0	629	491	858	1.2809	1203	349

3.6. The effect of ultrasonication on the molecular-weight distribution of humic acids analyzed by GPC

Since not all of the humic acid sample was dissolved in THF, the fraction detected by GPC is THF soluble and hereby referred to as THFS-humic acids. The GPC spectra of four THFS-humic acid samples are shown in Fig. 7. The weight-average molecular weights (M_W), number-average molecular weights (M_N), Z-average molecular weights (M_Z) and polydisperser indices (polydispersity) of the humic acids are summarized in Table 3.

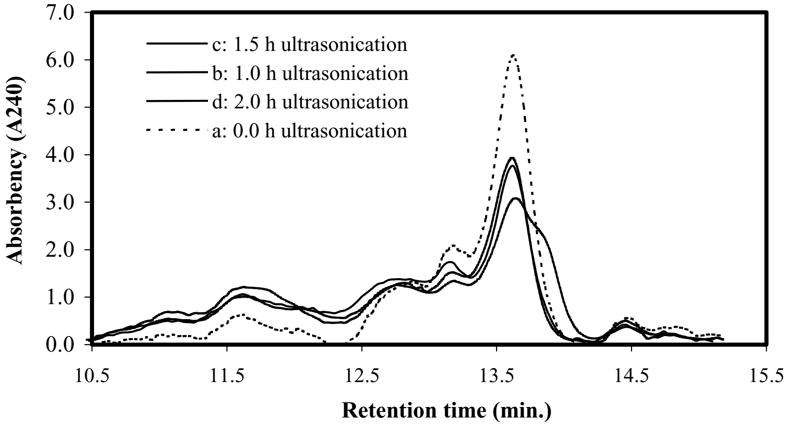

Fig. 7. Effects of ultrasonication on the molecular-weight distribution of the THF-soluble humic acids (GPC results, longer retention time suggests lower molecular weight).

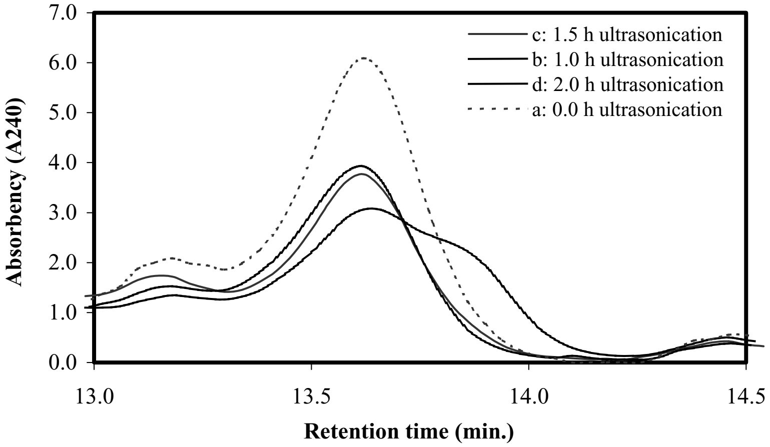

Fig. 8. Effects of ultrasonication on the dominant molecular fractions of the THF-soluble humic acids (GPC results, longer retention time suggests lower molecular weight).

The spectra show that one dominant peak at a retention time of 13.62 min and four minor peaks (retention time of 11.62 min, with molecular weight around 983; retention time of 12.78–12.86 min, with molecular weight around 500; retention time of 13.14–13.18 min with molecular weight around 430; and retention time of 14.46 min with molecular weight around 292) were detected in all the samples. In order to demonstrate the substantial changes in the spectra, detailed spectra with dominant absorbing areas are shown in Fig. 8. It is obvious that the peak areas decreased with ultrasonication time. A shift of the peak to a reduced molecular-weight area (with longer retention time) was observed in the sample exposed to 1 h of ultrasonication. It indicates that the dominant molecular-weight fractions of the tetrahydrofuran-soluble humic acids were effectively reduced by ultrasonication. However, the magnitude of the reduction did not constantly increase with ultrasonication time. Fig. 8 also shows that ultrasonication mainly affected the dominant molecular-weight fraction (intermediate molecular weight) of the humic acids. The relative increases in absorption intensities of spectra b, c, and d at retention times shorter than 12.5 min, compared with spectrum a, implied that some intermediate molecular-weight fractions of tetrahydrofuran-soluble humic acids might be aggregated into larger colloidal particles by ultrasonication.

Table 3 shows that for humic acids, $M_Z > M_N > M_W$ and polydispersity >1. The M_W of most THF-soluble humic acid molecules was below 338 Da. The M_N tends to be strongly influenced by lower molecular-weight components, whereas the M_W and the M_Z tend to emphasize the contribution of the heavier molecules in the mixture. The M_W and M_Z increased after ultrasonication, whereas the M_N showed little change after ultrasonication. This suggests that some heavy molecular particles were formed owing to the ultrasonication.

On the other hand, the higher the value of the polydipersity, the wider the range of molecular weight of the humic acids. Table 3 shows that the polydispersity of humic acids also increased after ultrasonication. This indicates that a wider molecular-weight composition range and some large colloidal particles of humic acids were formed in the ultrasonic field. This could be that when ultrasonication was applied on the polydispersed system of humic acids, the lower molecular weight (also the dominant) fraction was either decomposed or aggregated into large molecules. The larger and heavier molecular-weight component (minor fraction of the humic acids system) was difficult to break down and remained in the system, although it was expected that chain scissions near the center of the largest molecules could take place in an ultrasonic field [31].

Recently, Nikitenko et al. [33] have studied the sonolysis of diphenyl methane under the effect of 20 kHz ultrasound in argon at 60 °C. They have found that the sonolysis of diphenyl methane had caused the formation of a polymer with a composition similar to that of cross-linked polystyrene. The mechanism of diphenyl methane sonolysis was assumed to be that of diphenyl methane molecules dissociating inside the cavitating bubbles. Secondary radical scavenging and radical recombination yielded the sonopolymer in the liquid phase. Similarly, Atobe et al. [34] have found that uniform, dense films of electropolymerized aniline, pyrrole and thiophene could be formed under ultrasonication. Using a 38 kHz ultrasound field, they successfully speeded up the chemical polymerization of aniline, leading to the formation of conducting polyaniline colloids. These findings support the feasibility of chemical repolymerization of some dissociated fractures of the humic acid compounds in the ultrasound field.

In addition, Riera-Franco de Sarabia et al. [35] have proposed the acoustic agglomeration of submicron particles as a promising route towards enhancing the efficiency of filtration systems used for clarification. Therefore, besides chemical repolymerization, physical aggregation or flocculation could also affect the humic acids during ultrasonication.

3.7. The effect of ultrasonication on the morphology of humic acids

The effects of ultrasonication on the morphology of humic acids were investigated using SEM. As shown in Fig. 9(a), the humic acids consist of curved, closely-knit bundles of fibres and irregular sheet-like structures of varying thickness. The fibres often ended and started in protrusions extending from the plane of the sheet-like structures. Fig. 9(b)–(d) shows that the number of fibres quickly decreased and that they completely disappeared after 2 h of ultrasonication. However, the sheet-like structures became prominent, the thickness and orientation of the multi-layered structures with fine finger-like protrusions radiating from the surface were seen to be increasing after 2 h of ultrasonication, as indicated in Fig. 9(d). Fig. 9(b) and (c) shows that the flakes appeared to curl together at some points, forming multi-oriented structures with some fragmented open spaces and corrugated surfaces. Overall, it can be seen that the initial appearance of the humic acids was fibrous, consisting of bundles of fibres that were closely knit together or attached to sheet-like structures. After ultrasonification, the fibroid structures rapidly disintegrated and a closely-woven flake network structure resembling sponge and a thickened sheet structure with finger-like protrusions were frequently observed in the humic acids treated by ultrasonication. These results confirmed the effect that ultrasound had on the flocculation of the humic acids, as was suggested by the GPC results.

3.8. The effect of ultrasoniation on the E_4/E_6 ratios of humic acids

The effects of ultrasonication on the E_4/E_6 ratios of humic acids are also presented in Fig. 4. The changes in the E_4/E_6 ratios with ultrasonication were similar to the changes in the UV absorbency and FBB reactivity, increasing initially and then decreasing with prolonged ultrasonication. Under ultrasonication, the organic compounds in water were probably degraded via hydroxyl radical oxidation, pyrolytic degradation and supercritical water reactions. In the case of an aqueous solution, water vapour present in the bubble is split to yield $H^\bullet$ and $OH^\bullet$ radicals. The organic substrates present within the vapour phase or in the surrounding liquid of the collapsing bubbles are subjected to direct attack by $OH^\bullet$ radicals. Volatile compounds break up into the gas phase, i.e. into the gaseous bubble within the aqueous solution, and undergo direct pyrolysis.

With ultrasonication, the relatively weaker bonded alkyl side chains, long linear aliphatic side chains and C–O linked aromatic groups of the humic acids could be attacked preferentially and broken down into volatile compounds which

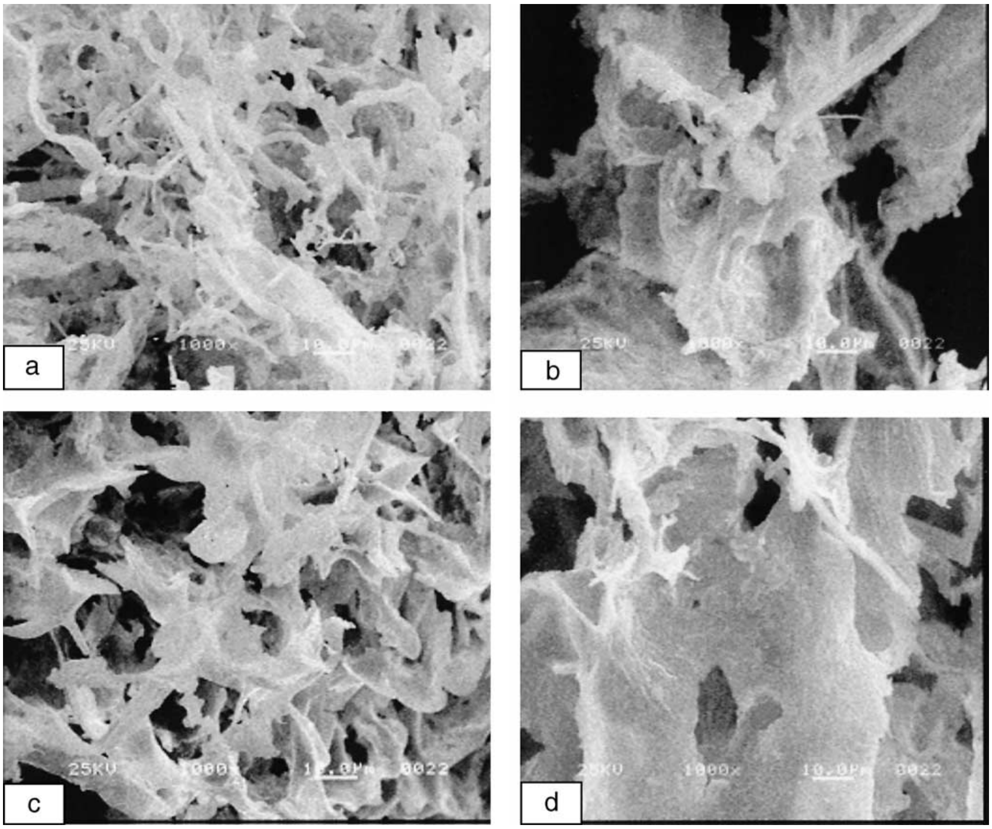

Fig. 9. SEM micrographs of humic acids (25 kV, magnification 1000×, bar = 10.0 μm) exposed ultrasonication over different periods: (a) 0 h ultrasonication; (b) 0.5 h ultrasonication; (c) 1.5 h ultrasonication and (d) 2 h ultrasonication.

could escape from the solution into the gas phase. Thus, the ratio of aliphatic carbon to aromatic carbon could be altered by ultrasonication. A good correlation between the changes in the UV absorbency and the FBB reactivity indicated that the increase of UV absorbency could initially be ascribed to the presence of 1-naphthol or other hydroxylated aromatic-like compounds in humic acid molecules. These compounds are in abundance at the early stages of ultrasonication, owing to the decomposition of aliphatic compounds. The decrease in UV absorbency could be ascribed to the decomposition of 1-naphthol or other hydroxylated aromatic-like compounds when ultrasonication was prolonged.

Furthermore, the UV absorbency was also affected by the macromolecular size and shape of the humic acids in the solution. It has been found that condensation (aggregation) of the humic acids molecules could be caused by ultrasonication. In view of the foregoing, the changes in UV absorbency could not be ascribed solely to changes in the chemical structure of the humic acid molecules, but also to changes in the physical configuration of the humic acids.

According to Ohta et al. [12], the E_4/E_6 ratio is inversely related to the degree of condensation of the aromatic network in humic acids. A low E_4/E_6 ratio would be indicative of a relatively high degree of condensation of aromatic constituents in humic acids, whereas a high ratio would reflect a low degree of aromatic condensation and the presence of a relatively large proportion of the aliphatic structure. Schnitzer and coworkers [23] suggested that light absorption of aqueous humic acid solutions in the visible region of the electromagnetic spectrum increased with: (i) the ratio of carbon in aromatic nuclei to C in aliphatic side chains, (ii) the total C content, and (iii) the molecular weight. Later, Chen et al. [6] suggested that much of the observed visible absorption by humic acids may be due to light scattering, which thus may contribute to the lowering of the E_4/E_6 ratio in the high molecular-weight fraction of humic acids. Secondly, the E_4/E_6 ratio of humic acids is primarily governed by particle sizes and weights, and is apparently not related to the amount of condensed aromatic rings in the structure. Thirdly, the effect of pH on absorption and E_4/E_6 ratio is due to the changes in particle size possibly caused by folding or unfolding, or aggregation or dispersion of the humic acid macromolecules.

The results in this study show that the average molecular weight of humic acids increased with the aggregation or flocculation of the low molecular-weight fractions of humic acids after ultrasonication. Chemical changes were detected in a variety of aliphatic carbons (mainly polymethylene), β-aliphatic carbon on the aromatic rings, α-carbon oxygen bond and carboxylic acid groups or α-methylene groups on the aromatic rings. However, it is difficult to draw a definite correlation between the chemical structural changes, as well as molecular-weight changes of humic acids and the changes of E_4/E_6 ratios of humic acids. Therefore, the changes in E_4/E_6 of the humic acids with the period of ultrasonication could be owing to both the changes in the aromatic to aliphatic ratios of humic acids and the aggregation or dispersion of the humic acid macromolecules caused by ultrasonication. Overall, the effect of ultrasonication on humic acids could be explained by two mechanisms. One is the chemical degradation of humic acids in the cavitation bubbles by oxidation. The other is the physical aggregation of humic acid fragments, which suggests that ultrasonication can be used as a pretreatment for the filtration of aqueous humic acid solutions.

4. Conclusions

- In the polydispersed humic acid system, the lower molecular-weight fraction (dominant fraction) of the humic acids was affected by the ultrasonication, and was decomposed into a volatile form and evaporated from the system. The larger molecular-weight fraction (minor fraction) tended to aggregate into a colloidal form and remained in the system.
- The chemical modifications induced by ultrasonic irradiation mainly occurred in the carboxylic acid groups, the α-methylene groups on the aromatic rings, a variety of aliphatic carbons (mainly polymethylene), β-aliphatic carbon on the aromatic rings and α-carbon oxygen bond.
- The changes in E_4/E_6 ratio in the humic acid solutions could result from both the changes in the aromatic to aliphatic ratio of humic acids and the aggregation or dispersion of the humic acid macromolecules caused by ultrasonic irradiation.
- SEM micrographs showed that the initial humic acids appeared as fibres and bundles of fibres that were closely knit together or united, with a sheet-like structure. With ultrasonication, the fibrous structures decreased rapidly, and the aggregation of a closely-woven flake network structure resembling sponge and a thickened sheet structure with finger-like protrusions was observed.
- The effects of ultrasonication on the humic acids could be explained by two mechanisms. One is the chemical degradation of the humic acids in the cavitation bubbles by oxidation. The other, the physical aggregation of humic acid fragments.

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