Biodegradation 7: 287–295, 1996.

Spectroscopic and elemental investigation of microbial decomposition of aquatic fulvic acid in biological process of drinking water treatment

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Accepted, 19 April, 1996

Key words: aquatic fulvic acid, biological treatment of drinking water, ^{13}C-NMR spectroscopy, infrared spectroscopy, microbial decomposition, UV-Vis spectroscopy

Abstract

As humic substances left in treated water tend to form trihalomethans during chlorination, their removal in water treatment processes is a significant concern for drinking water supplies. One of the removal technologies, the biofilm reactor is studied for the microbial decomposition of aquatic fulvic acid (AFA). The AFA is characterized by elemental analysis, UV-Vis, ^{13}C-NMR, and IR spectroscopic methods. The spectroscopic and elemental investigation was capable of characterizing the microbial decomposition of AFA. Biologically treated fulvic acid was in a more oxidized state; its spectra displayed a higher degree of condensation of aromatic constituents than influent fulvic acid. Microbial degradation of AFA was more active in the low molecular weight fractions and intensively occurred in the aliphatic fraction.

Abbreviations: A_λ – the absorbance at wavelength; α_λ – specific absorptivities; AFAs – aquatic fulvic acids; AHS – aquatic humic substances; COD – chemical oxygen demand; Da – dalton; DO – dissolved oxygen; E_4/E_6ratio – a ratio between absorbance at 465 and 665 nm; FA – fulvic acid; IR – infrared; NMR – nuclear magnetic resonance; TOC – total organic carbon; UV-Vis – ultraviolet-visible

Introduction

Humic substances are natural organic matter and play important roles in terrestrial and aquatic environments. Humics constitute 25% of total organic carbon on earth and 50% of organic carbon in oceans and fresh waters (Aiken et al. 1985). In the last decades, progress has been made in understanding the properties and behavior of aqueous humic substances. In the 1950s, they were still described as 'Yellow Acids' by Shapiro (1957), but further studies of their composition, functional groups, structures and reactivities, etc., were reported (Rebhun et al. 1993). The humic substances can be divided into humic, fulvic, and hymathomelanic acids based on their hydrophilic properties. The humic acids are soluble in alkaline solution, but precipitate in strong acid solutions. The fulvic acids (FA) are soluble over the entire pH range and typically account for 80–90 percent of the humic substances in surface waters (van Breemen et al. 1979). Aquatic humic substances (AHS) are polar and straw-colored organic acids that are derived from soil humus, as well as terrestrial and aquatic plants. In lakes, up to 80% of dissolved organic carbon (DOC) is dissolved humic substances. Recently, there has been increasing concern over removing organics as well as turbidity from surface waters (Edzwald et al. 1987), because some of the humic substances contained in treated water are precursors of trihalomethanes, which cause serious problems to human health (Rook 1977). AHS are sources of methyl groups in the production of chlorinated methanes in water treatment. The other roles of humic substances in water chemistry include high affinity for trace metal ions and organic pollutants, such as pesticides and herbicides. Hence the humic substances should be thoroughly removed in the water purification process.

In many cases, conventional physicochemical treatment processes are ineffective for the removal of organic contaminants including humic substances, while biological processes could be a viable alternative. Until now, only a few studies have been reported on the biodegradation of AHS (Shapiro 1957; De Haan 1974, 1977; Stabel et al. 1979; Rittmann 1985). Even though humic substances are often considered relatively refractory, they could be degraded by some microorganisms. The microbial decomposition of humic substances (Rittmann 1985) and the isolation of an *Arthrobacter* sp. which was grown slowly on the humus as the only carbon source (De Haan 1977) support the hypothesis.

The main purpose of this study is to compare the composition of aquatic fulvic acid (AFA) isolated from the raw water with that from the biologically treated water by using Amberlite XAD-8 resin. The characterization techniques used to reveal differences between isolated samples of AFA were:

- elemental analysis,
- ultraviolet-visible (UV-Vis) spectroscopy,
- infrared (IR) spectroscopy, and
- ^{13}C-nuclear magnetic resonance (NMR) spectroscopy.

In addition, AFA samples were fractionated by ultrafiltration according to Aiken (1984) to determine the molecular size distribution.

Materials and methods

Isolation and fractionation of fulvic acids

The AFAs were extracted from coagulation/precipitation (C/P) sludge, which was obtained from a water treatment plant and a biofilm reactor effluent. They were named *FAi* and *FAe*, respectively. The biofilm reactor was fed with the *FAi*, extracted from the C/P sludge, as the only carbon source.

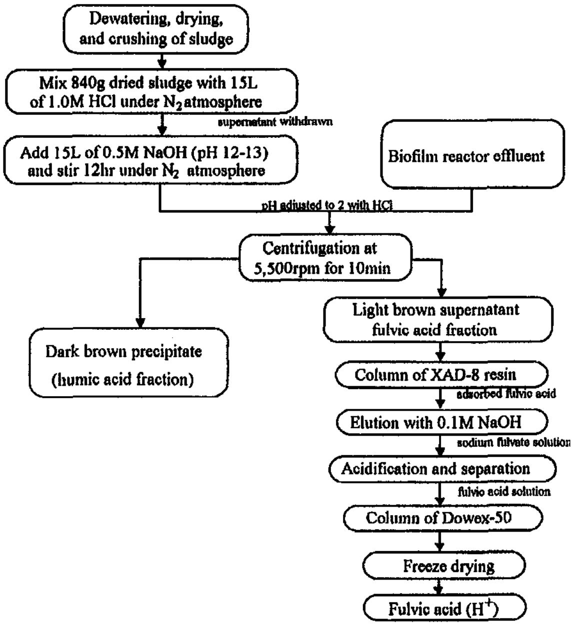

Figure 1. Schematic description of fulvic acid extraction and purification procedure.

The *FAi* was extracted and purified as described in Figure 1 according to the reported procedure (Nash et al. 1980). The mixture of air-dried sludge of about 840 g and 15 l of 1.0 M HCl was shaken for 16 h at room temperature to decompose free carbonates and to remove alkaline earth metals. The residue separated from the supernatant by centrifugation at 4050 ×g for 5 min was neutralized to pH 7 using 1 M NaOH. Subsequently, 15 l of 0.1 M NaOH was added to the residue and followed by slow stirring under N_2 atmosphere for 24 h. The residue was separated from the dark-colored supernatant by centrifugation. The above procedures were omitted in case of *FAe*. The FA fraction was separated by acid precipitation at pH lower than 2 with 6 M HCl, leaving the humic acid fraction in the sediment. The FA fraction was isolated from the supernatant by sorbing it on a column of purified XAD-8 resin.

The FA was eluted from the column with 0.1 M NaOH (flow rate of 2–3 ml/min) followed by the addition of NaF to the solution and left overnight to remove trace silicate. After adjusting the pH of the solution to 1.0, the FA was adsorbed to XAD-8 resin once again. The resin was washed with 0.1 M HCl to remove the remaining trace impurities and then eluted with 0.1 M NaOH. This sodium fulvate solution was analyzed to know the molecular size distribution. The sodium salt of FA was then passed through a cation-exchange column of Dowex 50X (H^+) to remove Na^+. This protonated final product of FA was freeze-dried.

All works were implemented in vessels made of materials free of plasticizer (polycarbonate) to avoid organic contamination. These vessels were soaked in dilute acid bath for one day and thoroughly rinsed with distilled water prior to use. The Amberlite XAD-8 resin

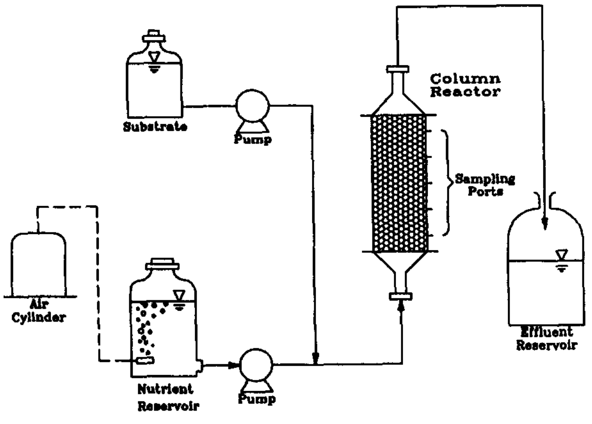

Figure 2. Scheme of the laboratory-scale reactor for the removal of fulvic acid.

used for the isolation of AFA was cleaned by Sohxlet extraction in series with acetonitrile, diethylether, and methanol for 24 h, respectively. The resin then was rinsed with tertiary distilled water. After drying in a 60 °C oven, the resin was packed in a 2.5 × 50 cm glass column and protonated with 0.1 M HCl.

Reactor for biological degradation

Biological processes used in drinking water treatment are of the biofilm type, their detention time is as short as a few minutes (Rittmann & Snoeyink, 1984). In this study, small-scale biofilm reactors were designed to stimulate a nitrification filter, as shown in Figure 2. The glass column reactor of 5 cm diameter and 20 cm high was filled with 3-mm glass beads. Two peristaltic pumps were used for feeding at a constant flow rate so as to keep hydraulic retention time at 30 min. Dissolved oxygen (DO) in the effluent was maintained over 5 mg/l.

As a unique organic carbon source, *FAi* of 3 mg/l as TOC was fed with 8.5 mg/l KH_2PO_4, 21.8 mg/l K_2HPO_4, 17.8 mg/l Na_2HPO_4, 1.5 mg/l KNO_3, 11.0 mg/l $MgSO_4$, 27.5 mg/l $CaCl_2$, 0.15 mg/l $FeCl_3$ $6H_2O$ and 1.0 mg/l $NaHCO_3$. To avoid bacterial growth outside the reactor, end caps, inert polymer screens which hold the beads in the column, and the tubings were sterilized and thoroughly rinsed with distilled water whenever the feed reservoir was emptied. Supernatant from settled activated sludge was passed into the bead-filled column for several detention times and stopped to allow the inoculum to contact the beads for 24 h. After 24 h, the influent and effluent tubings were sterilized, and the column was connected to the influent reservoir.

Analytical methods

Total Organic Carbon (TOC)

TOC of the aqueous sample was measured using a TOC analyser (Rosemount Analytical Inc. model DC-180). As TOC is the sum of purgeable organic carbon (POC) and nonpurgeable organic carbon (NPOC), the POC is first measured and then the NPOC is measured through UV/persulfate reaction of all carbon to carbon dioxide followed by detection of CO_2 in an IR analyser. The uncertainty in this assay (quoted as a standard deviation) was less than 2% of the average measured value for the range of TOC concentrations. Suspended solids were removed from samples by centrifugation prior to TOC analysis.

Elemental analysis

C, H, and N contents are analyzed by burning 25 mg of FA in an O_2 atmosphere, and the resulting CO_2, H_2O and N_2 (reduced from NO_2) gases are analyzed by a heat conductivity measurement with CHN Rapid instrument (Heraeus Co.). The oxygen content in the samples is analyzed by a cracking process which oxidizes carbon to CO then to CO_2 over I_2O_5. The CO_2 was quantified by titration. Sulfur is analyzed by oxidizing the sample in H_2O_2 under O_2 atmosphere and by titrating H_2SO_4 acid produced with $Ba(ClO_4)_2$.

Molecular size distribution

The molecular size distribution of FAs is determined by using ultrafiltration. In a typical experiment, 25 ml of sodium fulvate solution was diluted to about 240 ml with deionized water, and the pH was adjust to 8.0 with 0.01 M HCl solution. This solution was diluted to a final volume of 250 ml. The experimental setup consisted of a stirring filtration cell of 10 ml (Product No. 8010, Amicon Co.), which is connected to the 25-ml sampling vial. The filters of different pore sizes used in the experiment were YM100, XM50, YM10, YM5 and YM2 (Amicon Co.). The filters were washed with distilled water and 0.1 M NaOH solution prior to use. The experiment was run under N_2 gas pressure of 413.7 kPa (60 psi). Constant stirring was used to avoid the accumulation of solute molecules on the membrane surface. Membrane performance was evaluated by measuring the absorbances of the initial solution and filtrate at 254, 350, 465, and 665 nm, followed by TOC analysis. A detailed procedure for the FA characterization is given elsewhere (Aiken 1984).

UV-Visible absorbance

Absorbance measurements were made at 254, 350, 465 and 665 nm in 0.025 M $NaHCO_3$ (Chen et al. 1977) on a spectrophotometer (Beckman DO-70), using 1.0 cm cells. From these measurements, specific absorptivities relative to the TOC concentration, α_λ in m^2/g, were calculated by the following equation.

$$\alpha_\lambda = {}^{A_\lambda}/_{(L \times TOC)}$$

where, A_λ is the absorbance at the wavelength, L is the path length of the cell in meters, and TOC is in g/m^3. The E_4/E_6 ratio was calculated from absorbance measurement in triplicate at 465 and 665 nm, respectively.

Infrared spectroscopy

The infrared spectra were recorded on KBr pellets (1 mg of FA per 100 mg of KBr) in a BOMEM mode MB 100 FT-IR spectrophotometers. KBr (FT-IR Grade, Aldrich Co.) was dried by heating and cooling down in a desiccator. A good representative absorbance spectrum was obtained with a scan of 10. A linear baseline correction function was applied to each spectrum using 3,800 cm^{-1}, 2,000 cm^{-1}, and 860 cm^{-1} as zero-absorbance points to facilitate spectrum comparison.

^{13}C-NMR spectroscopy

The ^{13}C-NMR spectra were recorded using a Bruker AM 300 instrument operating at 75.47 MHz. Sample solutions were prepared by dissolving approximately 120 mg FA in 1.0 M NaOD followed by shaking for several hours, centrifuging at 3800 rpm for 15 min and filtering through glass wool into 10 mm NMR tubes. The conditions were chosen for obtaining quantitative spectra with the decoupler off except during the acquisition time of 0.2 s. A pulse width of 45°, and the total delay time of 2.0 s were chosen to minimize distortions due to the nuclear Overhauser enhancement (nOe) effect on different carbon types and lack of complete relaxation of all carbons. Around 3×10^4 FIDs (free induction decays) were average signal of each spectrum, and the line broadening function of 50 Hz was applied to enhance the ratio of signal to noise. After Fourier transformation, the baseline of the spectrum was modified using the Bruker software to match and subtract parabolic functions. The uncertainty of the measurements in the peak area was approximately $\pm$ 8%, depending on the extent of spectral resolution and baseline distortion.

Results and discussion

Substrate utilization

For about 1 month, 3 mg C/l of *FAi* was fed to get a steady-state biofilm in the reactor. This corresponded with a volumetric loading rate of 144 mg C/l day. Mineralization of *FAi* increased to about 10% after two weeks feeding, then stayed at about 13% throughout the rest of the experiment. A biofilm layer developed at the lower part (first 3 cm) of the reactor and moved slowly along the column with time. As the *FAi* was the only electron donor in the feed, its oxidation should provide the carbon and energy sources for long-term biofilm growth and maintenance. Figure 3 shows the dissolved organic carbon (DOC) and DO concentrations in the influent and effluent of the biofilm reactor from the 40th to the 75th day of operation. The concentrations of DOC in the influent and the effluent were relatively constant, resulting in constant removal of DOC. On the other hand, Figure 3 shows that the removal of DO increased slightly with time. This might be due to endogenous DO consumption by the biofilm in the reactor and to partial oxidation of *FAi*. A relatively high DO consumption, an average of 1.2 mg/l, caused by total DOC removal of 0.4 mg C/l compared well with the theoretical amount of 1.07 mg/l for full oxidation of the *FAi*. Because the DOC removal data give information of the complete mineralization of *FAi* to CO_2, partially oxidized carbon was not included in DOC removal, although it consumed DO. From this view, more than 0.4 mg C/l might have been partially oxidized.

Elemental composition

The elemental compositions (C, H, N and O) and COD/TOC ratios of the AFA samples are given in Table 1. Two AFAs analyzed have elemental composition data that fall within the ranges reported in the literature (Steelink 1985), except for higher content of nitrogen in *FAi* and of oxygen in *FAe*. Unusually high nitrogen content in *FAi* indicates the local-specific feature of FAs.

In general, the H/C and O/C ratios are useful indicators for the structural changes of AFA, which might have occurred in the biofilm reactor. The *FAe* is distin-

Table 1. Elemental composition, molar ratios and COD/TOC ratio of fulvic acids.

Sample	weight %				Atomic ratio			
	C	H	N	O	H/C	O/C	N/C	COD/TOC
FAi	46.5	4.7	5.3	43.5	1.204	0.702	0.098	2.800
FAe	27.3	2.0	1.5	69.2	0.873	1.196	0.047	2.406

Ash-free basis.

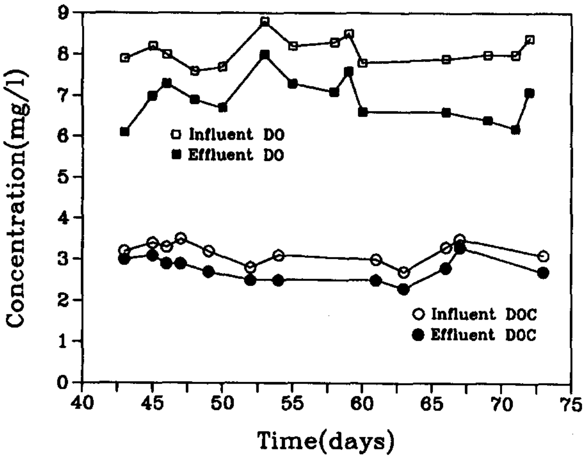

Figure 3. DOC and DO in reactor influent and effluent from the 40th day to the 75th day.

guished by a lower H/C and an increased O/C ratio. The lower H/C ratio of *FAe* indicates an increased aromaticity or unsaturation degree. The presence of aliphatic groups including primary amino groups usually gived rise to a H/C larger than one (Steelink 1985). The higher H/C ratio of *FAi*, therefore, could be caused by abundant nitrogen present in the form of amino groups. The COD/TOC ratio, which is the ratio of oxidizable components of the AFA (mainly C, H and S) to total organic carbon content, decreases as H/C ratio decreases, indicating partial biological oxidation of FA in the biofilm reactor. The increased O/C ratio, i.e. from 0.7 to 1.2, also implies that the oxidation state of *FAe* is higher than *FAi*. Another possible reason of the increased O/C ratio is a low carbohydrate content in *FAe* (Thurman & Malcolm 1983), since carbohydrates may be easily degradable.

Molecular size distribution

The molecular size distribution of FA was determined by an ultrafiltration technique. The fraction of two FAs left on filters, as well as molecular weight cut-off of ultrafilters, are shown in Table 2. It shows that *FAi* has more high-molecular fractions than *FAe*, indicating *FAe* is made up of smaller molecules. While 14.8% of TOC in *FAi* was captured by the ultrafilter XM50 (molecular weight cut-off of 5,000 Da), only 1.6% of TOC in *FAe* was retained. This increase of the low molecular weight fraction resulted from the partial decomposition of *FAi*.

Table 2. Molecular size distribution pattern of fulvic acid samples.

Molecular weight range (Dalton)	% of Total organic carbon	
	FAi	*FAe*
< 1,000	26.9	46.9
1,000– 5,000	58.3	51.5
5,000– 10,000	3.7	0.2
10,000– 50,000	3.3	1.2
50,000–100,000	6.5	0.1
> 100,000	1.3	0.1

Spectroscopic characterization

UV-Visible spectra

As shown in Table 3, specific absorptivities (α_λ) increase as the FA pass through the biofilm reactor, indicating an increase in aromaticity of FA. According to Flaig et al. (1975), specific absorptivities of soil humic substances generally increase with increasing phenolic hydroxyl content. In this report, the increase of phenolic content was confirmed by the NMR spectra of the samples as discussed later.

The E_4/E_6 ratio, that is the ratio of absorbance of 465 to 665 nm, has been widely used for characterization purposes (Stevenson 1982; Chen et al. 1977). In this study, the E_4/E_6 of *FAe* was higher than that of *FAi*. Chen (1977) proposed that the E_4/E_6 ratios of humic and fulvic acids were governed primarily by particle sizes and weights. The molecular size of *FAe* was smaller than that of *FAi* (see Table 2), which account for the high E_4/E_6 ratio of *FAe*.

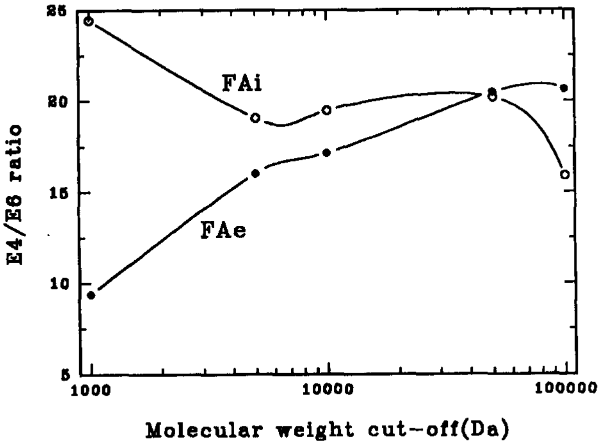

Figure 4. Variation of E_4/E_6 with molecular weight cut-off of fulvic acids.

For additional information on the relationship between molecular weight and the E_4/E_6 ratio, E_4/E_6 ratios of five FA fractions were determined. Figure 4 shows that molecular weight of *FAi* had a distinct effect on the E_4/E_6 ratio. The lowest molecular weight fraction had the highest ratio, and vice versa. In case of *FAe*, however, the E_4/E_6 ratio increased the molecular weight, indicating that the microbial degradation of FA was more active on low-molecular weight FA fractions (Stabel et al. 1979). The large molecular weights in the natural polymers could retard mass transport to and within the biofilms more than smaller solutes, such as carbohydrates, amino acids, and carboxylic acids. The study on biofilm removal kinetics of humic and fulvic acids indicated that one of major factors retarding removal of large-molecular polymers was the decreased diffusion rate (Rittmann 1985). Hence, the lower molecular weight fractions became more aromatic and this decreased the E_4/E_6 ratios of these fractions. The E_4/E_6 ratio decreased with increasing molecular weight and condensation, which could be an index of humification (Stevenson 1982). Thus, a low ratio may indicate a relatively high degree of condensation of aromatic constituents, while a high ratio suggests a low degree of aromatic condensation and more aliphatic structures.

IR spectra

Figure 5 shows the specific absorption bands of FA observed in all the IR spectra. The strong absorption bands exist in regions typically associated with humic substances, as follows: 3400 cm^{-1} (H-bonded OH), 2900 cm^{-1} aliphatic C-H stretching), 1600–1660 cm^{-1} (aromatic C = C and ionized carboxylate groups), 1420 cm^{-1} (C-H bending of CH_2 or CH_3 groups and C-O stretching of phenolic OH), and 1030–1280 cm^{-1} (ethers, esters, and other C-O groups). The IR spectra features of two AFAs were similar to the previous study (Stevenson & Goh, 1972). The band intensity in the 1600–1660 cm^{-1} range is rather weak with a center near 1640 cm^{-1}, while the band intensity near 1720 cm^{-1}, indicative of C = O in COOH, is quite weak; the latter is different from other FAs.

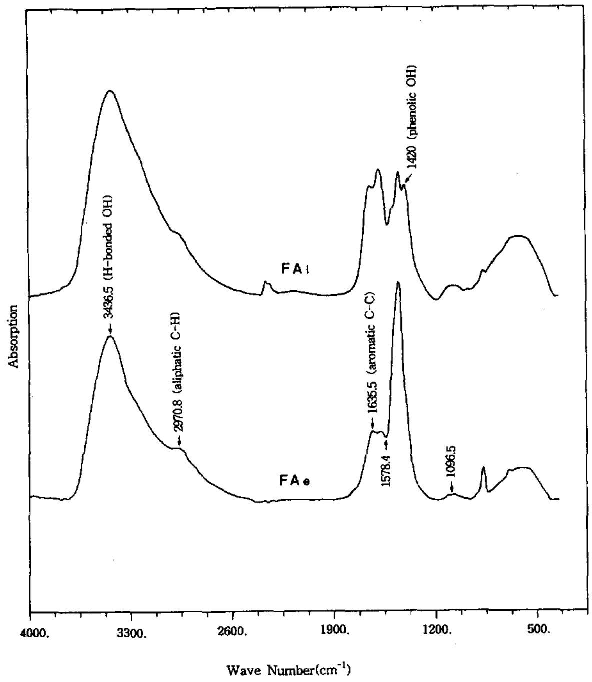

Figure 5. Infrared spectra of the *FAi* and *FAe.*

According to the IR spectra of both FA samples, *FAe* tends to exhibit weaker absorption at 1650, 1580, 1420, and 1096 cm^{-1}. The decrease in absorbance at 1580 and 1096 cm^{-1} indicates that microorganisms attack aliphatic chains, such as amino acid and polysaccharide groups. The decrease in remained absorbance at 1650 and 1420 cm^{-1} indicates the decrease of the aromatic fraction. In relation to substrate utilization as described previously, decrease in absorbance at 1580 and 1096 cm^{-1} could relate to removal of the DOC that is most easily degradable, while the decrease at 1650 and 1420 cm^{-1} could be associated with excess DO consumption due to partially oxidized carbon. The changes in IR absorbance therefore suggest that the microorganisms in the biofilm could use all fractions of FA as organic substrates. The decrease of aromat-

Table 3. Specific absorptivity, TOC and E_4/E_6 ratio of fulvic acid samples.

Sample	α_λ (m^2/g)				TOC (mg/l)	E_4/E_6
	254 nm	350 nm	465 nm	665 nm		ratio
FAi	2.8060 (1.9218)	0.6334 (0.4338)	0.0793 (0.0543)	0.0063 (0.0043)	68.49	12.63
FAe	3.9684 (1.4572)	0.9221 (0.3386)	0.1114 (0.0409)	0.0065 (0.0024)	36.72	17.04

Data in parenthesis are absorbances at wavelength λ.

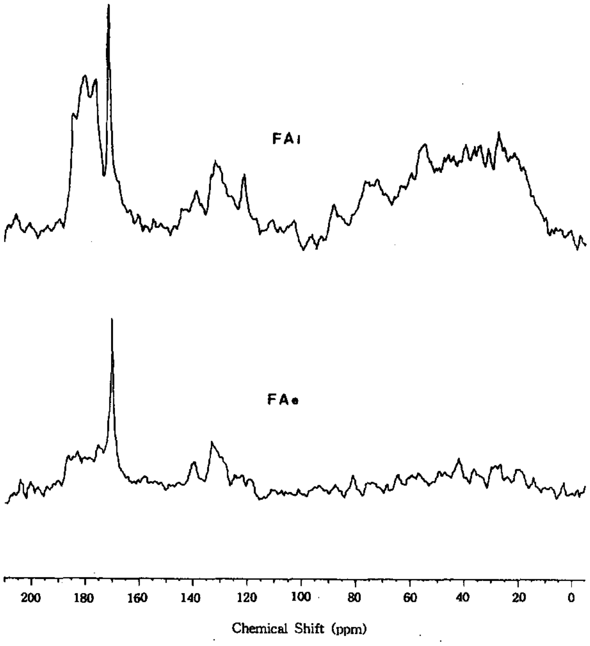

Figure 6. ^{13}C-NMR spectra of the *FAi* and *FAe*.

ic compound associated with FA might result from a decomposition or ring-opening of aromatic compounds, such as phenolic group and benzene derivatives. In natural surface water, benzoic acid having structural resemblance to FA is degraded by the β-ketoadipate pathway, in which the ring-opening step is comprised (Stanier & Ornston 1973).

^{13}*C-NMR spectra*

As shown in Figure 6, the ^{13}C-NMR spectra are similar in appearance to that of most AFAs, as reported previously (Heizlar et al. 1994). These spectra contain distinct peaks in both aliphatic (0–105 ppm) and aromatic (105–165 ppm) regions. In the *FAi*'s spectrum, the peaks at 27 and 31 ppm might be aliphatic carbons of alkyl chains (Hatcher et al. 1980; Shin et al. 1994). The strong peak at 31 ppm is the long alkyl $(CH_2)n$ chains, while that at 40 ppm may include contributions from alkyl carbons and amino acid carbons. The resonance at 72 ppm is caused probably by polysaccharides. The wide range of aromatic resonances (105–150 ppm) centered around 131 ppm are observed, which indicates a variety of substituents of the aromatic rings. The peak at 131 ppm is assigned as the alkyl-substituted aromatic carbons and aromatic CH (Shin et al. 1994; Inbar et al. 1990). The resonances between 174 and, 190 ppm may be assigned to carbon atoms in carboxyl and in amide groups. These resonances are not particularly strong in the ^{13}C-NMR spectrum of the *FAe*, in contrast with the spectra of *FAi* and of soil FA, where the carboxyl C resonance is the most prominent feature (Shin et al. 1994). The sharp peak at 171 ppm may be the carbonate anion (CO_3^{-2}), which could have arisen from carbonate originally in the extraction solution, or during the preparation and storage of NMR samples (Preston & Schnitzer 1987). In contrast to the *FAi*, the *FAe*'s spectrum displayed broad resonances in the aliphatic regions and sharper bands in aromatic regions. The bands in the region 0–105 ppm and 174–190 ppm are reduced more than those in the other fractions. These bands have been attributed to long-chain aliphatic moieties and carboxyl groups and are positive evidence for showing that the microbial decomposition of AFA was centralized in the aliphatic fractions. In other words, the aliphatic fraction associated of the FA appeared to have a greater microbial affinity. This might be caused by weak association, such as a hydrogen bond between carbohydrates or amino acid and body of FA (De Haan & De Boer 1978).

The assignments for major bands in these NMR spectra are given in Table 4. Integration of areas under the bands in the decoupled spectra allows estimation of relative contents according to the six regions outlined in Table 4. The division of the spectra into six regions is somewhat arbitrary. The uncertainties were estimated by making measurements in triplicate. The relatively

Table 4. Relative intensities of various carbons in fulvic acids determined by ^{13}C-NMR spectra.

Chemical shift regions (ppm)	Assignment	Relative intensities (%)	
		FAi	*FAe*
(0– 47)	aliphatic carbons	32.7 ± 0.6	17.3 ± 1.2
(47–105)	alcohols, amines, carbohydrates, acetates	24.6 ± 0.4	11.5 ± 1.2
(105–145)	aromatic carbons	15.0 ± 0.1	21.6 ± 1.7
(145–165)	phenolic carbons	2.8 ± 0.2	8.5 ± 0.4
(165–174)	carbonate anion	6.2 ± 0.1	29.5 ± 2.7
(174–190)	carboxyl carbons	18.5 ± 0.4	11.6 ± 1.5

high intensities in the aromatic region suggest that *FAe* have undergone more aromatic condensation than the *FAi*. These ^{13}C-NMR data support the previously mentioned findings that indicate an increase in aromaticity from *FAi* to *FAe* and reduced aliphatic fractions.

Conclusions

Biological processes have the potential to remove AFA effectively from natural water supplies. Biologically treated fulvic acid is in a more oxidized state, and its spectra displayed a relatively high degree of condensation of aromatic constituents than influent fulvic acid. Microbial degradation of AFA was more active in the low molecular weight fractions and intensively occurred in the aliphatic fraction.

The spectroscopic investigations, together with elemental analysis, appear to be useful means of characterizing the microbial decomposition of AFA in terms of the aromaticity and oxidation state of the fulvic acids. While an appreciable amount of data have been accumulated concerning their composition, structures, and physico-chemical control methods, information regarding their degradative biochemical pathway is lacking. This research showed that AFA could be biologically degraded or stabilized. However, further work is needed in order for a better understanding of the process involved.

Acknowledgments

The authors would like to thank Mr. Seok Woo Lee for his technical assistance. This work was supported financially by the Korea Sanhak Foundation and the Keo-bo Industrial Co. Ltd.

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